Recent Developments in Solid Phase Microextraction Techniques

Master of Science Thesis

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Tiivistelmä/Referat – Abstract

The literature part of this thesis consists of a review of recently introduced forms of solid phase microextraction (SPME): thin film microextraction (TFME), in-tube solid phase microextraction (IT-SPME) and the closely related techniques of capillary in tube adsorption trap/solid phase dynamic extraction (INCAT/SPDE). The experimental part covers the study of reagents for on-fiber derivatization of low molecular weight aliphatic amines in atmospheric concentrations.

In TFME a thin film of sorbent is used for extraction instead of a rod-like sorbent as in fiber-SPME. This increases analyte uptake and capacity compared to fiber-SPME, making TFME suitable for non-equilibrium extraction. TFME is used with both gas and liquid chromatography, although the large size of the film presents problems in desorption, especially in gas chromatography. Common applications of TFME are environmental monitoring and in vivo extraction.

IT-SPME is a dynamic type of SPME most often coupled with liquid chromatography, in which a liquid sample is pumped through an extraction capillary. It is relatively easily automated with most autosamplers. In the most common form a sorbent is coated on the inside walls of the capillary. Recently, packed types of IT-SPME have been introduced, which can achieve very high extraction efficiencies. In addition, sorbent materials which change their properties according to environmental factors such as temperature, potential and magnetic field seem promising for future development

INCAT/SPDE utilizes internally coated metal needles for extraction. Although similar to IT-SPME, it is used for sampling gaseous compounds by pumping them through the needle. Desorption and analysis is usually performed with a gas chromatograph. INCAT/SPDE has some advantages over fiber-SPME, such as larger sorbent volume and robustness. However, it is currently limited to only polydimethylsiloxane-based sorbents, which limits possible applications.

In the experimental part, the possibilities of using allyl isothiocyanate, pentafluorobenzaldehyde (PFBAY) and pentafluorobenzyl chloroformate (PFBCF) in simultaneous extraction and on-fiber derivatization of low molecular weight aliphatic amines were explored. Separation and analysis was performed with gas chromatography-mass spectrometry. Allyl isothiocyanate did not derivatize the analytes. On-fiber derivatization with PFBAY was successful for both ethylamine and methylamine, but the concentrations required to observe signal from the derivatives were too high to use PFBAY for air samples. PFBCF was identified as the most promising reagent, working for both dimethylamine and ethylamine. It was also possible to construct a calibration function for gaseous dimethylamine.

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thin film microextraction, in-tube solid phase microextraction, solid phase dynamic extraction, on-fiber derivatization

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Table of Contents

Abbreviations

CAR	carboxen
CE	capillary electrophoresis
DART	direct analysis in real time
DESI	desorption electrospray ionization
DVB	divinylbenzene
EI	electron ionization
ESI	electrospray ionization
FID	flame ionization detector
GC	gas chromatography
INCAT	in-tube capillary adsorption trap
IT-SPME	in-tube solid phase microextraction
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
MALDI	matrix-assisted laser desorption/ionization
MIP	molecularly imprinted polymer
MS	mass spectrometry
MW	molecular weight
PAH	polyaromatic hydrocarbon
PAN	polyacrylonitrile
PDMS	polydimethylsiloxane
PEEK	polyether ether ketone

PFBAY	pentafluorobenzaldehyde
PFBCF	pentafluorobenzyl chloroformate
PLOT	porous layer open tubular
PPy	polypyrrole
RAM	restricted access material
SBSE	stir bar sorptive extraction
SCX	strong cation exchange
SPDE	solid phase dynamic extraction
SPE	solid phase extraction
SPME	solid phase microextraction
STE	sorptive tape extraction
TDU	thermal desorption unit
TFME	thin film microextraction
UV	ultraviolet
WAX	weak anion exchange
WCOT	wall coated open tubular

1. Introduction

During the last few decades most of the development in analytical chemistry has been driven by rapid improvements in instrumentation. This eventually led to the development of sophisticated instruments which coupled gas chromatography and liquid chromatography to mass spectrometry. These instruments have become increasingly common to the point that they are now considered nearly ubiquitous in analytical chemistry laboratories worldwide.

However, despite the sophisticated instruments now available, sample pretreatment initially did not undergo the same rapid development. Although advanced, GC-MS and LC-MS still require pretreatment of the sample, such as removal of matrix components or pre-concentration. The lack of development in pretreatment caused a situation where very advanced instrumentation was combined with pretreatment methods that have been in use since 19th century, such as LLE and Soxhlet extraction. These require manual labor and use large amounts of solvents. Due to these reasons, it is justified to say that sample pretreatment is a bottleneck in nearly all analytical methods, and the greatest gains in sample throughput can be made by shortening the pretreatment process.

Extraction, the removal of compounds of interest from the sample matrix, is an important part of the sample pretreatment process. The most common way to do this is to bring another phase in contact with the sample matrix, which will cause the transfer of analytes into the extracting phase. Usually, it is preferred that the extraction is exhaustive, meaning that all the analytes will transfer into the extracting phase. Although the transfer of compounds between the phases is always dependent on a thermodynamic equilibrium, with suitable selection of the extracting phase and its volume, as well as the volume of the sample, an extraction can be made nearly exhaustive in practice. Non-exhaustive extraction is generally called equilibrium extraction.

Microextraction is defined as an extraction method where the amount of extracting phase is very small compared to the sample volume. [1] The extracting phase can take many forms, but the most common types are solid sorbents or liquids. Because of the small amount of the extracting phase compared to the sample, a common feature of all equilibrium microextraction techniques is that they usually extract only a small amounts of analytes in the sample, hence the name microextraction. Perhaps the most well-known example of microextraction is fiber solid phase

microextraction (fiber-SPME), which was first introduced by Arthur and Pawlizsyn in 1990. [2] Although there are also exhaustive types of microextraction, [3] all SPME methods are equilibrium-based, which can be thought as their defining feature. Microextraction is a very diverse and quickly developing field, and there is no commonly agreed way to classify the many developments in the field during the last two and a half decades. In addition, sometimes techniques with different names are quite similar in principle, which causes additional confusion.

As microextraction has become a very popular field, it is not possible to review all microextraction methods in a single thesis. Therefore, a closer look will be taken to some of the recent developmens in SPME: thin film microextraction (TFME) and in-tube solid phase microextraction (IT-SPME). In addition, the closely associated techniques of in-tube capillary adsorption trap (INCAT) and solid phase dynamic extraction (SPDE) are also reviewed. Exhaustive microextraction techniques are not covered in this thesis, nor are any of the microextraction methods which use liquid as the extracting phase.

The experimental part concerns with on-fiber derivatization in fiber-SPME. In this type of fiber-SPME, derivatization is done directly on sorbent of the SPME fiber either during or after extraction. This allows, for example, the derivatization of analytes which are permanent gases. The target analytes were small aliphatic amines, which typically have low extraction efficiency due their high volatility. In addition, most amines have poor gas chromatographic properties, which is the reason why even heavier amines are often derivatized. Therefore, on-fiber derivatization was hoped to both reduce the volatility of the amines, increasing extraction efficiency, as well as improve their chromatographic properties at the same time.

2. Solid phase microextraction

2.1 Equilibrium extraction

In any type of system composed of two phases in contact which each other, partition equilibrium will be formed between them. This principle is behind of almost all chemical extraction techniques. Such a system can be, for example, a sorbent rod placed in a gaseous sample matrix as in headspace fiber-SPME or two liquid phases as in LLE. A general equilibrium equation can be written describing the distribution of a compound between the phases (Equation 1). [4]

$$K_D = \frac{C_2^{eq}}{C_1^{eq}}$$ (Equation 1)

Where c_1^{eq} and c_2^{eq} are the equilibrium concentrations of the compounds in phase 1 and 2, respectively. Here K_D is called the distribution constant. When K_D value is small, the compounds will mostly stay in phase 1, and when K_D value is high, they prefer to move to phase 2.

In SPME, the extracting phase is usually exposed to the sample until equilibrium is reached between the extraction phase and sample. If only two phases are included, like when the extracting phase is placed in a completely gaseous sample, it is possible to write the following equation. [4]

$$c_0 V_s = c_s^{eq} V_s + c_p^{eq} V_p$$ (Equation 2)

In Equation 2, c_0 is the initial concentration of the analyte, V_s and V_p the volumes of the sample and extracting phase respectively and c_s^{eq} and c_p^{eq} the equilibrium concentrations of the analyte in the sample and on the extracting phase. Now, if one takes phases 1 and 2 in Equation 1 and assigns it as the sample matrix (phase 1) and sorbent (phase 2), Equations 1 and 2 can be combined to Equation 3 to obtain the equilibrium concentration in phase 2.

$$c_p^{eq} = c_0 \frac{K_D V_s V_p}{K_D V_p + V_s}$$ (Equation 3)

It is simple to convert concentration on the fiber to the amount of substance (marked n), as shown in Equation 4.

$$n = c_p^{eq} V_p = c_0 \frac{K_D V_s V_p}{K_D V_p + V_s}$$ (Equation 4)

In microextraction, the volume of the extracting phase is usually very small compared to the sample volume. [4] Therefore, it can be assumed that V_s is very large compared to V_p, which also makes the term $K_D V_s$ very small compared to V_s. By rewriting Equation 4, a new simplified equation is obtained:

$$n = K_D V_p c_0$$ (Equation 5)

From Equation 5, it can be determined that the volume of the sample does not need to be known, as long as its volume is very large compared to the volume of the extracting phase. It is also evident that the volume of sorbent has direct relationship with the amount of analyte molecules that can be extracted.

The exact time to achieve equilibrium can be estimated from Equation 6 if the diffusion constant of the analyte is known. [4]

$$t_{95\%} = \frac{3(b-a)}{2D_f} \quad \text{(Equation 6)}$$

In this equation, $t_{95\%}$ refers the time required to reach 95% of equilibrium concentration of analyte. b-a refers to the thickness of the extraction phase and D_f is the diffusion coefficient of the analyte. It can be seen that increasing phase thickness can lead to much longer equilibration times. Therefore, even for analytes with very large diffusion coefficients it can take a long time to equilibrate with a thick phase.

2.2 Exhaustive extraction

In exhaustive extraction, the term K_D is very large, making the term $K_D V_p$ much larger than V_s. In this case, Equation 3 can be reformulated to Equation 7, which implies that all the analyte in the sample will be collected in to the extraction phase. [4]

$$n = V_s c_0 \quad \text{(Equation 7)}$$

Exhaustive extraction is rare in SPME as it is only suitable for analytes with a high K_D value. Furthermore, the capacity of the extraction phase is limited, so only small sample amounts can be exhaustively extracted. Exhaustive types of microextraction, such as needle trap or in-tube extraction, use much larger amounts of sorbent to achieve exhaustive extraction.

2.3 Solid phase microextraction in capillaries

There are types of SPME where the extraction phase is bound on the inside walls of a tube, such as in-tube SPME (IT-SPME) and solid phase dynamic extraction (SPDE). For these types of SPME the estimation of extracted analyte amount is not simple. Concentration profile along lengthwise (x-axis) along the capillary as a function of time (t) can be estimated from Equation 8. [4]

$$c(x,t) = \frac{1}{2} c_0 \left[1 - \mathrm{erf}\left(\frac{\frac{x-ut}{1+k_p}}{\sigma\sqrt{2}}\right)\right] \quad \text{(Equation 8)}$$

In this equation u is the velocity of the fluid or gas through the capillary and k_p is the partition ratio:

$$k_p = K_d(V_p/V_v) \quad \text{(Equation 9)}$$

Where V_v is the void volume of the capillary. The σ in Equation 8 is square root of dispersion front:

$$\sigma = \sqrt{Ht\frac{u}{1+k_p}} \qquad \text{(Equation 10)}$$

H is height equivalent to theoretical plate. As can be seen, Equation 8 is quite complicated and almost never applied in practice. Furthermore, it only applies in extraction systems where the sample is directly in contact with the sorbent – for headspace extraction systems the partitioning between the sample matrix and headspace also must be taken into account. To my knowledge, there is no mathematical model to describe SPME in packed capillaries. Nevertheless, there are some observations indicated in Equation 10 that can be useful. [4] Analytes migrate along the length of the capillary with a speed which is proportional to the sample velocity. However, the speed of the analytes is inversely proportional to their partition ratios, i.e. their speed is slower if they have higher affinity to the sorbent. Thus, the speed in which sample is pumped though the capillary has an influence on the extraction efficiency.

2.4 Calibration in solid phase microextraction

Classic calibration methods, such as external standard calibration curve, standard addition and internal standard are all applicable in SPME. With non-exhaustive SPME, there are several possibilities to realize these calibration methods, depending on which part of extraction profile one wants to use. A typical extraction profile in SPME is shown in Figure 1.

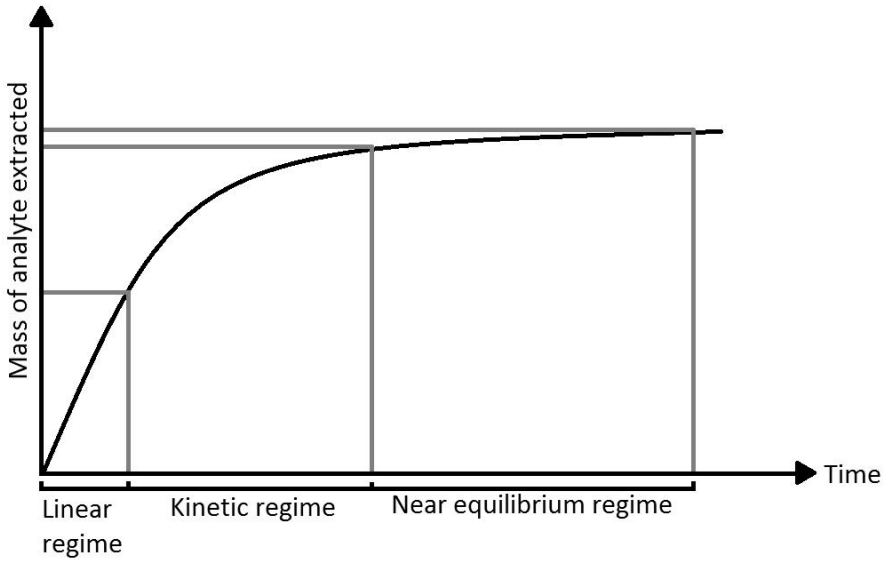

Figure 1. Typical extraction profile in SPME. Figure redrawn from [5].

The extraction profile can be roughly divided in two main components: kinetic regime and near equilibrium regime. From the kinetic regime a linear regime, where extracted analyte amount has

nearly a linear relationship with extraction time, can be further separated. Any of the regimes can be used for calibration, although calibration within the equilibrium regime is the traditional approach to SPME. As it maximizes the amount of extracted analytes and is relatively easy to understand, equilibrium calibration remains popular. However, more recently, calibration methods based on the kinetic regime have also been studied. The strength of kinetic calibration is that it there are models available that do not require a calibration curve made under similar conditions as the actual extraction, which is a necessity in equilibrium calibration. They do require the determination of additional factors, however.

Kinetic calibration approaches without an external calibration curve depend on the estimation of speed of mass transfer between the extracting phase and sample matrix. For example, an extracting phase can be moved in the sample matrix at a known rate, which allows a determination of sampling rate coefficient (R_s). [6] This in turn enables the calculation of concentration of the analyte in the sample if the mass of the analyte in the extracting phase can be determined (Equation 11).

$$m_{analyte} = c_s R_s t \qquad \text{(Equation 11)}$$

The sampling rate is different for each SPME device and must be experimentally established. The main drawback of this type of kinetic calibration is that it requires constant and known agitation of the sample in order for the sampling rate to be comparable between samplings.

To remove the need for constant agitation in kinetic calibration, on sorbent standard method was introduced by Chen et al. [7] In this type of kinetic calibration approach, a standard is loaded on the extracting phase before exposing it to the sample matrix. The standard desorbs at a similar rate as the analyte adsorbs, and essentially functions as an internal standard, correcting for differences in extraction conditions. The analyte concentration in the sample can be calculated from Equation 12:

$$c_s = \frac{s_0 n}{K_d V_p (s_0 - s)} \qquad \text{(Equation 12)}$$

In equation 12, s_0 is the initial concentration of the standard, s the concentration of the standard after the extraction and n the extracted analyte amount. On sorbent standard calibration requires much less knowledge on the extraction conditions and no agitation, but the on sorbent standard has the same problem as internal standards in general. That is, it should be chemically similar, but still separable from the analyte. On sorbent standard also should not be present in the sample matrix, as this will affect the desorption properties compared to the analyte, which is initially not present on the fiber.

It should be noted that most of these calibration types are mainly applicable to only passive types of SPME, such as fiber-SPME and TFME, as the dynamic techniques do not have mathematical models to support kinetic calibration. For these types of SPME, it is often enough to stop the extraction at a point where sufficient amount of analytes have been extracted for successful analysis or equilibrium has been reached.

3. Thin film microextraction

Thin film microextraction (TFME) was introduced by Bruheim et al. in 2003. [8] One can recall from Equation 5 that it is possible to extract larger amounts of analytes with a large volume of the extracting phase and increase the overall analysis sensitivity. However, according to Equation 6, equilibration time increases with phase thickness. Therefore, in order to achieve optimal geometry for an extracting phase, its surface area to volume ratio should be maximized. TFME takes advantage of this by replacing the tube-like sorbent used in fiber-SPME with a thin film, as a cube has a larger surface area to volume ratio than a cylinder.

The first introduced TFME devices used 1 cm^2 and 2 cm^2 PDMS films attached to a deactivated stainless steel rod, as shown Figure 2. [8] This film was used to extract PAHs from spiked water samples in both headspace and direct immersion modes. When extracting, the thin film was spread to resemble a flag supported by the stainless steel rod (Figure 2). For desorption, the PDMS film was wrapped around the rod so that it fit inside a GC injector liner. Since then, many different types and shapes of thin film have been introduced. They can be for example squares, pentagons, circles or blades. They can be made completely out of the extracting phase, or can be inert material coated with sorbent.

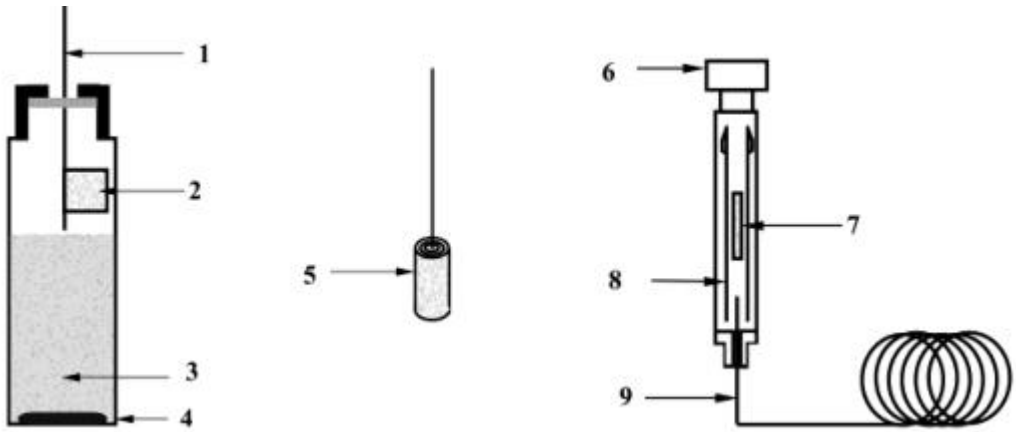

Figure 2. First TFME system described. 1. Deactivated stainless steel rod, 2. PDMS thin film, 3. aqueous sample, 4. magnetic teflon stirring bar, 5. thin film in desorption mode wrapped around a stainless steel rod, 6. GC injector nut, 7. approximate location of thin film in desorption mode, 8. 54 x 5.0 x 3.4 mm glass liner insert and 9. GC colum. [8] Reprinted with permission from American Chemical Society.

3.1 Extraction parameters in thin film microextraction

TFME is essentially fiber-SPME with different sorbent phase geometry, and works under the same principles. Therefore, the parameters that affect TFME are the same as in fiber-SPME. However, the large volume of sorbent sometimes needs to be taken into account.

According to Equation 5, doubling the volume of the extracting phase should double the amount of compounds extracted. However, it can be seen from Figure 3 by Bruheim et al. that for most of the analytes, doubling of the extracted amount cannot be reached with any of the analytes with a double-sized 2 cm^2 thin film compared to 1 cm^2. [8] This was thought to be because it is usually assumed in microextraction that the sorbent volume is negligible compared to the sample volume (see Equation 5) and that the analyte will partition equally to all of the extracting phase. These assumptions begin to break down as the size of the thin film is increased, and the extracted amount begins to deviate from a linear relationship with the volume of extracting phase. In addition, the due to the large volume of the extracting phase, it was possible to extract a significant fraction – roughly 30% value was given for acenapthylene – of the total analyte amount. Therefore, the large extracting phase may have altered the concentration of analyte, especially in the gas phase, from which the extraction occurred. This likewise altered the extracted amount. Similar effects were also observed by Qin et al. [6] To avoid problems such as these, in large extraction phase microextraction it is important to pay attention to the volume of the sample.

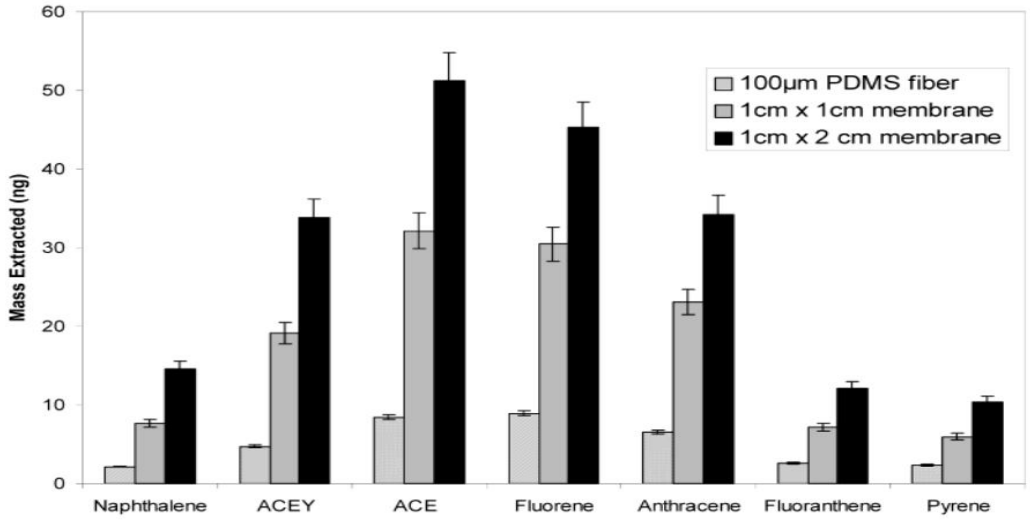

Figure 3. Headspace extraction efficiency of PAHs compared between fiber-SPME, 1 cm^2 TFME and 2 cm^2 TFME. Sample matrix was 20 mL of spiked water in a 40 mL closed vial, extraction time was 60 minutes in 30 °C. Analysis with GC-MS. ACEY is shortened for acenapthylene and ACE for acenapthene. Error bars represent standard deviation of three repetitions. [8] Reprinted with permission from American Chemical Society

3.2 Thin film microextraction compared to other solid phase microextraction methods

Due to the optimized geometry, under identical conditions TFME can potentially extract higher amounts of analytes in a shorter time than fiber-SPME. [6, 9-11] As an example, Qin et al. compared the performance of TFME to fiber-SPME in kinetic calibration with a known sampling rate (Figure 4). [6] Under the same conditions, TFME was able to extract much more analytes from the same sample.

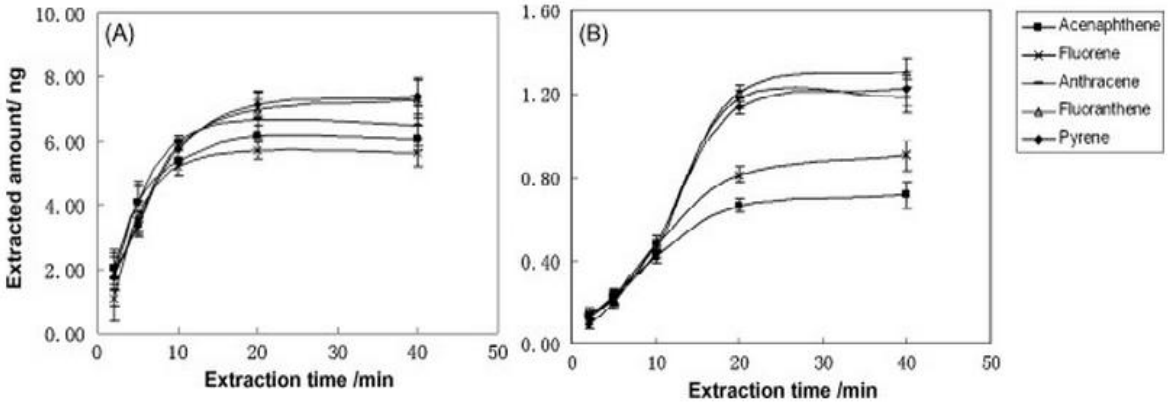

Figure 4. Comparison of extraction profiles of PAHs from water with TFME (A) and fiber-SPME (B). Both extraction phases were PDMS which were rotated 600 rpm with a portable electric drill. The fiber was rotated off-center in a circular motion. Analysis with GC-MS. Error bars represents standard deviation of three repetitions. [6] Reprinted with permission from Elsevier B.V.

Qin et al. also compared TFME to SBSE in the extraction of PAHs from river water, [12] as shown in Figure 5. Twister is brand name for a commercial SBSE device, which was compared to a self-made PDMS TFME. The equilibration time for SBSE is extremely long, as Twister uses sorbent phases between 500 μm and 1000 μm thick, and in fact equilibrium was not reached even in 400 minutes. This can be compared to 127 μm film used for TFME by Qin et al. [12] Furthermore, the extracted mass with TFME was more than double, even if Twister extraction was continued to 400 minutes.

Although the limitations of TFME compared to other types of SPME have not been yet thoroughly investigated, the results that are available seem promising. TFME has larger capacity and faster sample uptake than fiber-SPME but it does not suffer from long equilibration times like SBSE.

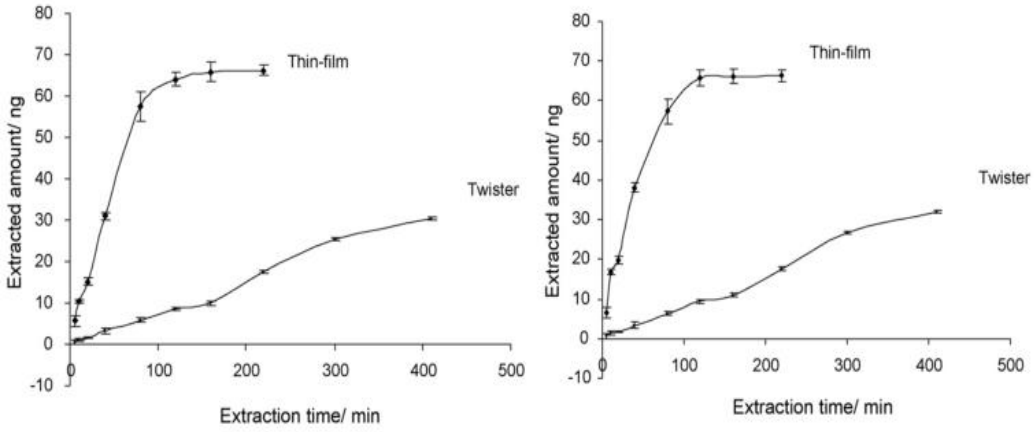

Figure 5. Comparison of TFME and commercial "Twister" SBSE device in direct immersion extraction of PAHs from a 1L river water sample. Left: fluoranthene, right: pyrene. The extraction phase was PDMS in both cases. Analysis with GC-MS. Error bars represent standard deviation of three repetitions. [12] Reprinted with permission from Elsevier B.V.

One possibility is also to combine several different types of extraction methods to obtain a better profile of a complicated sample. This approach was adopted by Eom et al. for investigation of indoor air in a room infested with the common bed bug. [13] Needle trap, fiber-SPME and TFME were all utilized in determining what kind of volatile organic compounds are associated with bed bugs. The fiber-SPME and TFME were made of different sorbent materials to complement each other. Needle trap provided the best coverage of compounds detected in the sample. As the TFME was made out of PDMS, it discriminated highly polar and volatile compounds, while having the best extraction efficiency for less volatile compounds. Fiber-SPME material was PDMS-DVB-CAR, so it had better affinity for polar and volatile compounds.

3.3 Kinetic calibration in thin film microextraction

Although equilibrium calibration is the most popular method of calibration with TFME, the properties of thin films make them attractive for kinetic calibration. The large surface area and faster analyte uptake compared to fiber-SPME counteract the lower amounts of analytes extracted compared to equilibrium calibration. For example, Qin et al. used kinetic calibration with a known sampling rate by attaching a PDMS thin film to an electric drill in two studies (Figure 6). [6, 12] The agitation speed could be adjusted by controlling the speed of the drill, which made it possible to determine the sampling rate (see Equation 11). By using kinetic calibration, Qin et al. were able determine low nanograms per liter levels for most PAHs with an extraction time of only eight minutes with the improved method. [6]

Figure 6. On-site extraction system for PAHs. The PDMS thin film is under the water, rotated by the electric drill. [6] Reprinted with permission from Elsevier B.V.

There were some observations worth of noting. Firstly, Qin et al. noticed that repeatability was improved when the extraction was allowed to proceed closer to equilibrium. [12] Secondly, it should be ensured that the thin film remains rigidly in shape if any kind agitation is used with TFME. If the thin film begins to bend or deform, the sampling rate will change and the analyte uptake will be slower. [12] In the second article the issue of thin film bending was solved by copper mesh support. [6]

On sorbent standard calibration has also been used with TFME. Bragg et al. used a PDMS thin film as a passive sampling device for PAHs from natural waters. [10] On-fiber standard calibration was found to have satisfactory results, although due to the long sampling time of one month, equilibrium was occasionally reached. Oyuang et al. later used the same technique in extraction

PAHs from different water depths. [9] PDMS TFME proved to be a simple, cheap and sensitive passive extraction device.

On sorbent standard calibration has also been used in in vivo extraction, as the nature of sampling from living organisms places limits on the extraction time and making a calibration curve may be difficult due to the complicated sample matrix. For example, Togunde et al. used the calibration method to determine pharmaceutical compounds from muscle tissue of live fish after exposure to wastewaters. [11] Bessonneau et al. used on-fiber standard to determine various compounds from saliva both ex vivo and in vivo. [14] Both commented that on sorbent standard calibration was highly suitable for these types of samples.

3.4 Desorption

The geometry of the film presents challenges when GC is used to analyze the extracted compounds, and desorption is generally recognized as the biggest shortcoming in TFME. [15] Due to the small amounts of analytes extracted, microextraction is nearly always combined with splitless injection in GC. Unfortunately, splitless injection is very sensitive for desorption conditions and distorted or even split peaks can easily result from uneven desorption.

It is possible to wrap or bend flexible materials such as PDMS in order to fit the thin film it into an injection liner. [8, 16, 17] However, desorption is usually slow and uneven due the large size of the thin film, which is why thermal desorption units (Figure 7) have been utilized in many studies. [14, 18, 19] This is a modification of normal GC injection port designed for thermal desorption from larger extraction media. Normal GC injection ports, on the other hand, are designed for needles or needle-like devices. Thermal desorption units are capable for slow desorption, and typically feature a programmable heater for the insert where desorption is accomplished. For more volatile compounds which cannot be easily focused in the column there may also be some type of trap to focus the desorbed analytes into a sharper band before chromatographic separation. Partial automation has also been achieved, as some thermal desorption units are capable of transferring the desorption liner, where the thin film is placed, into the heater for desorption. However, extraction and insertion of the thin film into the desorption insert of the thermal desorption unit still have to be performed manually. [13]

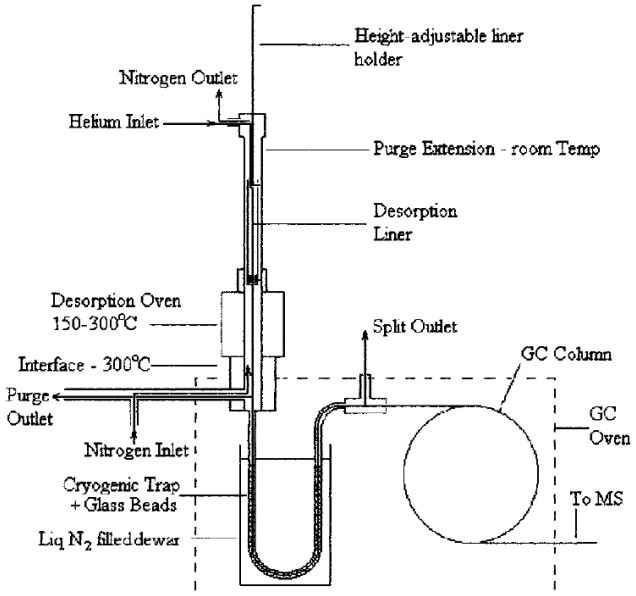

Figure 7. Thermal desorption unit with a cryofocusing device connected to a GC-MS. The desorption is achieved in the desorption liner, after which the desorbed analytes are focused in the cryotrap and released into the GC capillary as a narrow band. [20] Reprinted with permission from American Chemical Society.

If thermal desorption unit is not available, an alternative is to use a GC-compatible solvent to desorb the analyte molecules and inject the solution instead of thermally desorbing directly from the thin film. [16, 17] Naturally, this introduces an additional step into the analysis and increases usage of solvents. Furthermore, only part of the analyte molecules originally extracted on the thin film can be injected, as most GC systems cannot handle large amounts of solvents, potentially reducing sensitivity. In this case, one should consider if fiber-SPME could achieve similar results without the additional complications that solvent desorption required in TFME brings.

LC systems can handle much larger injection volumes than GC, in the range of tens of microliters compared to a few microliters for GC. Due to this, solvent desorption of TFME devices has been successfully combined with LC analysis in many cases. Solvent desorption is also easier to automate for high throughput formats than thermal desorption, as exemplified by the Concept 96 automated TFME system. [21] This sampler has fully automated extraction, desorption and analysis steps. It also includes automatic agitators for the samples. At the heart of the system are stainless steel blades coated with sorbent (Figure 8). On the other hand, to my knowledge, TFME-GC extraction and analysis has not been automated to this extent. The Concept 96 autosampler has been used for analysis of many types of samples. Some examples are biological fluids [22-24], sewage sludge [25] and phenolic compounds from wine and berry samples [26].

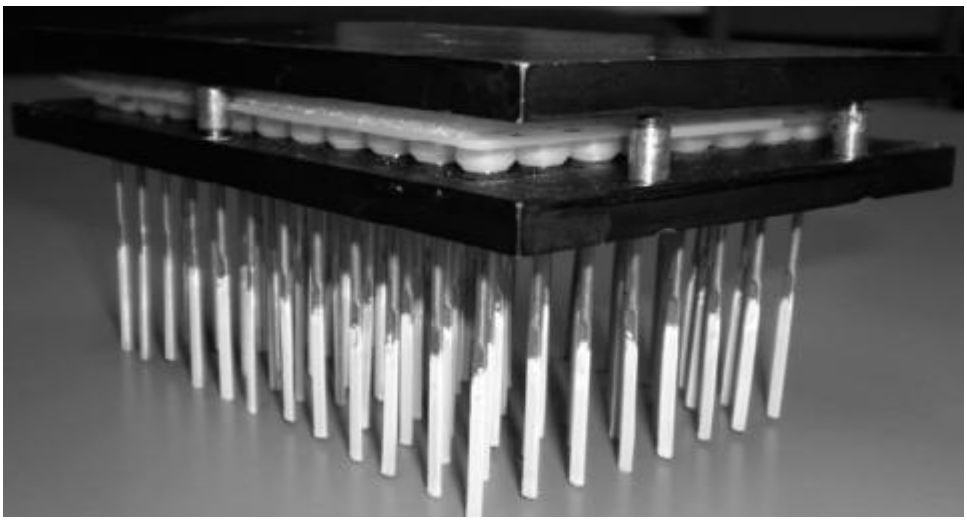

Figure 8. 96 blade TFME sampler. [27] Reprinted with permission from American Chemical Society.

One way to avoid the problems in desorption is to analyze the thin film directly without chromatographic separation. The flat shape of TFME makes it highly suitable for analysis types where a beam or spray has to be directed on the surface, as noted by Strittmatter et al. [28] By comparison, they are difficult to focus on the thin fiber used in fiber-SPME. Deng et al. for example, prepared a ZnO thin film on a glass sheet to determine sulphur dioxide content in wines. [29] Due to the selective nature of the material, surface-enhanced Raman scattering could be used in analyzing the thin film. The method was found to be both selective and sensitive, having limit of detection of 0.1 mg/L and producing similar results to a reference Monier-Williams method (oxidation of SO_2 to sulfuric acid and its titration) from real wine samples, although the repeatability of the TFME method was slightly lower.

In organic analysis He et al. manufactured a novel polystyrene/oxidized carbon nanotube sorbent for TFME. [30] It was used for headspace extraction of benzo[a]pyrene from water and urine samples, after which the thin film could be directly used as a matrix for MALDI-MS. The oxidized carbon nanotubes were found to be a good substrate material for MALDI, but required polystyrene attach the material on the MALDI plate, as the carbon tube material flew off from plate under vacuum. Limit of detection for benzo[a]pyrene was 50 ng/L from pure water, and recoveries in spiked water samples 81-123 %. [30]

Skipping the chromatographic separation in TFME applications has the potential for even higher sample throughput, especially when screening for a single compound. When it is necessary to identify multiple analytes from a single extracted sample, it is unlikely that a chromatographic separation step can be bypassed.

3.5 Different applications of thin film microextraction

Utilizing thin films in a similar manner to fiber-SPME, in either headspace or direct immersion sampling, has been TFME's most common application. However, due to the possibility of varying the geometry of the thin film and new coating methods, other extraction applications have also been investigated.

In vivo applications have already been noted. Togunde et al. studied in vivo the effect of pharmaceuticals in wastewater on trout. [11] C18-coated silica particles mixed with binder material were immobilized on metal strips and inserted into trout muscle tissue for 30 minutes and analyzed with LC-MS/MS. The results were also compared with fiber-SPME using the same sorbent. On sorbent standard kinetic calibration was used, so equilibrium was not reached. The mass transfer to and from the thin film was faster, giving TFME improved sensitivity over fiber-SPME. For example, the extraction rates (mass per time) from a gel spiked with carbamazepine, fluoxetine, ibuprofen and gemfibrozil were 2-3.5 times higher in TFME compared to fiber-SPME. Similar results were obtained from live fish muscle, where thin film extracted 2-4 times the mass compared to fiber in the same sampling time.

Jahnke et al. used pure PDMS thin films in a similar manner as Togunde et al. to investigate in vivo how lipid content affects the equilibrium calibration of polychlorinated biphenyls in fish tissue. [19]. In lipid-rich fish tissues, equilibrium was found to form quickly, in the matter of hours. On the other hand, in tissues containing less than 2% of lipids, equilibrium was not reached even with an extraction time of one week. Extraction times this long are not feasible in vivo, so kinetic calibration as utilized by Togunde et al. seems to be a more prudent approach.

In a different in vivo-application, there have been several studies of TFME-like technique called sorptive tape extraction (STE) in skin research. [31-34] This is very similar to PDMS-TFME, except that the PDMS thin film tape is attached directly to a test subject's skin. After extraction of the analytes from the skin, they were thermally desorbed and analyzed with GC-MS. Riazanskaia et al. used similar PDMS thin films to study the VOC profile of human skin [35], although they did not call this method sorptive tape extraction. A 0.45 mm thick PDMS film was simply cut to 1.5 cm x 2.0 cm pieces, cleaned and placed on test subjects' skin (Figure 9). After this they were covered and left for a determined time to extract the compounds, which were then analyzed with GC-MS. The chromatogram obtained from thermal desorption of the PDMS film was very complex, showing at least 300 resolved compounds, and many more unresolved ones. For several model compounds (2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid and heptanoic acid) the standard deviation of two in vivo extractions was less than 30%.

Figure 9. In vivo skin sampling with a PDMS thin film. [35] Reprinted with permission from Royal Society of Chemistry.

Later, Jiang et al. applied similar strategy for VOC sampling but also studied another approach of using a steel mesh to avoid direct contact of the PDMS thin film with skin. [36] Although it produced chromatograms with lower background, the signal intensity for analytes was also reduced. In addition, less volatile compounds were only detected with direct application of the PDMS film on skin, and the steel mesh may be a potential source of loss of analytes. [37] Although the chromatograms were cleaner, the repeatability of the in vivo extraction was similar to Riazanskaia et al. These results indicate that direct application of the PDMS film on skin may produce better results.

Apart from in vivo sampling, Golding et al. used vials coated with a thin film of ethylene-vinyl acetate to study the bioavailability of phenantrene in sediment. [16] The sample was inserted into a vial coated with the sorbent material and incubated for a set time. The vial was then emptied, rinsed with water and the analytes desorbed into an organic solvent, which was then analyzed with GC-MS. Golding et al. commented that ethylene-vinyl acetate thin film is a promising sorbent for bioavailability research, and it has found further use as passive samplers in monitoring other organic contaminants in water. [38, 39]

3.6 Thin film materials and coating procedures

PDMS was used in the first studies with TFME [8] and it still remains as a widely used adsorbent. It has many attractive properties: it is thermally stable, easy to desorb and clean out of most adsorbed compounds, biocompatible, resistant to many solvents, flexible and mechanically durable. In addition, it does not suffer from strong matrix effects even in complicated matrices, [40] making it robust choice for extraction of nonpolar compounds from different types of samples. The PDMS thin film can be purchased and cut into suitable shape to use in TFME. [10] It

is also possible to produce PDMS thin films in lab. [41] As can be seen from Table 1, PDMS thin films have very wide usability.

Table 1. Applications of PDMS in thin-film extraction.

Analyte	Matrix	Analysis	Reference
PAHs	Water	GC-MS	[6]
Polychlorinated biphenyls	Fish tissue	LC-MS	[19]
Pesticides	Water	Desorption corona beam ionization-MS	[42]
Volatile organic compounds	Skin	GC-MS	[36]
Volatile fraction, sebum	Herbs, skin	GC-MS	[31, 32]
Insect pheromones	Indoor air	GC-MS	[13]
Methyl jasmonate	Plant leaf tissue methanol-water extract	GC-MS	[43]
Traces of illicit drugs and explosives	Standards in various solvents	Ion mobility spectrometry	[44]

Despite the many positive qualities of PDMS, it cannot cover all extraction needs. The largest shortcoming of PDMS is that as a nonpolar material, affinities of polar analytes to it are generally low. Moreover, although PDMS discriminates polar compounds, it does not have much selectivity within nonpolar compounds, which can lead to difficulties in the analysis of complicated samples. To expand the applicability of TFME, new materials have been studied, and are they becoming more commonplace. These are listed in Table 2. Out of these, C18 on polyacrylonitrile support (PAN-C18) is perhaps the most used one.

Table 2. Applications of thin film microextraction without PDMS sorbent.

Material	Preparation	Analyte	Matrix	Analysis	Reference
C18 with polyacrylonitrile binder	Dipping, brushing and spraying on stainless steel blades	Benzodiazepines	Phosphate-buffered saline solution and human blood plasma	LC-MS/MS	[45]

Table 2 continues.

Material	Preparation	Analyte	Matrix	Analysis	Reference
C18 with polyacrylonitrile support	Spraying on stainless steel blades	Banned performance enhancing drugs	Urine, blood plasma	LC-MS	[22, 46]
Chemically modified cellulose paper	Dipping reagent solution vial	Estrogens	Water, wastewater, urine	LC with fluorescence detector	[47]
Cellulose paper with anticodeine aptamer	Chemical modification of cellulose paper, dipping to reagent vial	Codeine		Ion mobility spectrometry	[48]
Hydrophilic lipophilic balanced particles with polyacrylonitrile support or PDMS support	Spraying on stainless steel blades or mixing with PDMS	Prohibited substances	Saliva ex vivo (blades) and in vivo (PDMS mixture film)	LC-MS (blades), GC-MS (PDMS mixture films)	[14]
Nanostructured ZnO on glass	Dipping to reagent vial	Sulphur dioxide	Wine	Surface-enhanced Raman scattering	[29]
Tenax TA on zeolite support	Dipping to Tenax TA suspension	Volatile organic compounds	Standard solutions diluted with water	GC-MS	[49]
C18 with polyacrylonitrile support	Spraying on glass pane	Estrogens	Water	LC-UV	[50]

Table 2 continues.

Material	Preparation	Analyte	Matrix	Analysis	Reference
Polar enhanced phase sorbent with polyacrylonitrile support	Spraying on glass pane	Estrogens	Water	LC-UV	[50]
C18 particles with polyacrylonitrile support	Spraying on stainless steel blades	Bile acids	Bronchoalveolar lavage fluid	LC-MS/MS	[51]
Poly(vinylidene fluoride)	Purchased and cut	Endocrine disrupting compounds	Water	LC-UV	[52]
Mixed C18 particles and SCX on polyacrylonitrile support	Purchased commercially	Pharmaceutical and personal care components	Wastewater	DESI-MS	[28]
Polystyrene/oxidized carbon nanotubes	Electrospinning fibers on an aluminum plate	Benzo[a]pyrene and 1-hydroxypyrene	Water and urine	Used directly as a matrix for MALDI-TOF-MS	[30]
LC weak cation exchange particles with polyacrylonitrile support	Spraying on stainless steel blades	Rocuronium bromide and tranexamic acid	Blood plasma	LC-MSMS	[53]
Hydrophilic lipophilic balanced particles with polyacrylonitrile support	Spraying on stainless steel blades	Quaternary ammonium compounds	Water	LC-MSMS	[54]

Table 2 continues.

Material	Preparation	Analyte	Matrix	Analysis	Reference
C18 with polyacrylonitrile binder	Brushing on stainless steel mesh	Cocaine and methadone	Urine	DART-MSMS	[55]
Polystyrene-divinylbenzene with polyacrylonitrile binder	Spraying on stainless steel blades	Phenolic compounds	Wine, berry and grape	LC-MS/MS	[26]
Carboxen-PDMS and PDMS-divinylbenzene	Sping coating a glass wool mesh	N-nitrosamines	Water	GC-MS, thermal desorption unit	[18]

Mirnaghi et al. presented a method to coat stainless steel blades for use in the Concept 96 autosampler. [45] In this method, the blades were etched with hydrochloric acid and dried. After this, 5 μm C18 particles mixed with polyacrylonitrile in dimethylformamide was deposited onto the surface by spraying with high pressure and thermally cured. A total of ten layers were immobilized on the blades, which was stated to result in a phase thickness of 60 μm. Brushing and dipping methods were also tested, but the coating produced with these methods was unstable and peeled off the steel surface during use. Less than ten sprayed layers were also mentioned to have unsatisfactory durability. This method has since proven popular due to the stability of the coatings that can be produced. Furthermore, it is suitable for many different types of particles, [14, 28, 50, 53] and the polyacrylonitrile binder is highly biocompatible. [56]

Chemically modifying the surface of cellulose paper for TFME has also been done by simply dipping it into a reagent solution. Various common adsorbent phases used in SPE were successfully immobilized on cellulose paper by Saraji et al. [47] followed by an anticodeine aptamer by the same group. [48] Although only recently introduced for TFME applications, surface modification of cellulose has been extensively studied. [57] Therefore, this method is promising for even cheaper and easier preparation of custom-made thin films.

Another approach was introduced by Kermani et al. who used spin-coating on a glass wool mesh surface to manufacture thin films. [18] It was noted that any type of liquid-like material, possibly mixed with solid sorbents, can be used in the process. The thickness and properties of the thin films can be controlled better than in the previously mentioned methods. For example, it is possible to make layered thin films out of different sorbent materials. However, a dedicated spin-coating device is required for this kind of precision.

Along with these, other methods such as electrospinning [30] and sol-gel [58] have also been investigated. However, these methods have not yet found wide use in preparation of thin films for TFME.

3.7 Thin film cooling

Cooling the extraction phase increases the distribution coefficient, thus increasing the amount of analytes extracted. This is already a proven method to increase extraction efficiency in fiber-SPME, [59] and was recently introduced for TFME by Jiang et al. [60] The thermoeletric cooling device for a 102 μm PDMS thin film is shown in Figure 10. After extraction, a stick was used to push the thin film into the thermal desorption unit tube (TDU tube). Obviously, this type of system can only be used for thin films made of materials which are flexible and mechanically durable, like PDMS. Although portable, the cooling device is also quite large which makes it harder to handle the cooled TFME.

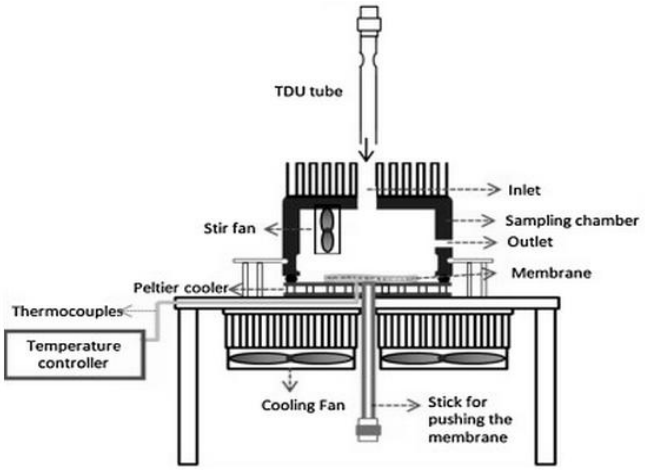

Figure 10. Thin film cooling device used by Jiang et al. [60] Reprinted with permission from Elsevier B.V.

Using a cooled thin film, Jiang et al. were able to extract nearly 2.5-fold amount of benzene in 5 °C compared to 23 °C at equilibrium. [60] However, it was noted that although cooling increases the

distribution coefficient and the amount of analyte that can be extracted, it increases the time required to reach equilibrium.

3.8 Trends in thin film microextraction

TFME seems to be following general trends of fiber-SPME. In recent years, the general focus has been bioanalysis, especially in vivo applications (Tables 1 and 2). Important part of this is the development of high throughput TFME analysis, such as the Concept 96 autosampler. Due to the varied geometry available, TFME can be applied to many types of extraction formats. For example, Bessonneau et al. developed a chewable PDMS-based sorbent for determining prohibited substances from saliva. [14] Like fiber-SPME, TFME is highly portable, so another area where it has found many applications is on site environmental sampling. On site and in vivo samplings have also been combined. [11]

Similarly to fiber-SPME, development of new sorbent materials for TFME has also intensified during the recent years. As the methods for coating sorbents on thin films are becoming more routine, experimenting with new sorbents should become easier and faster in the near future. As mentioned before, some attempts have been made at developing sorbent materials which are selective enough that chromatographic separation is not required.

Unlike in fiber-SPME or IT-SPME, using physical phenomena such as temperature, electricity or magnetic fields to exert more control over the extraction process have not been yet much investigated. Only one study investigating cooled thin film is available to my knowledge. [60] It remains to be seen if it is possible to manufacture, for example, magnetically or electrically sensitive thin films.

It can be seen from Tables 1 and 2 that while most applications of PDMS thin films have been combined with GC, new novel phases are mostly developed for use with LC analysis. Thin film desorption in GC requires a special desorption system, such as a thermal desorption unit, and there is no commercial fully automated TFME system for GC. Furthermore, thermal desorption in GC typically involves high temperatures, which already limits the type of materials that can be used. On the other hand, liquid desorption does not require any expensive modifications or can be done completely automatically. With the exception of thin films made of PDMS and without new ways to formulate TFME more suitable for GC, it seems that TFME will move to a mostly LC-based technique.

4. In-tube solid phase microextraction

In-tube solid phase microextraction (IT-SPME) is a type of SPME where the extracting phase is inside a capillary, through which the sample moves. The most common type of IT-SPME is a hollow capillary, where the extracting sorbent is placed around the internal wall (Figure 11A). It resembles WCOT GC capillary and in fact GC capillaries are commonly used in IT-SPME. In the study introducing the technique, Eisert and Pawilzyn used a piece of Omegawax 250 WCOT GC column to extract benzene and phenylureas from aqueous samples. [61] An important point is that IT-SPME was already completely automated in this first study through modification of a commercial LC autosampler. In addition to open capillaries, packed capillaries were also introduced to improve extraction capacity, efficiency and selectivity. The packed capillaries can be divided in three main types: particle packed (Figure 11B), fiber packed (Figure 11C) and monolithic (Figure 11D).

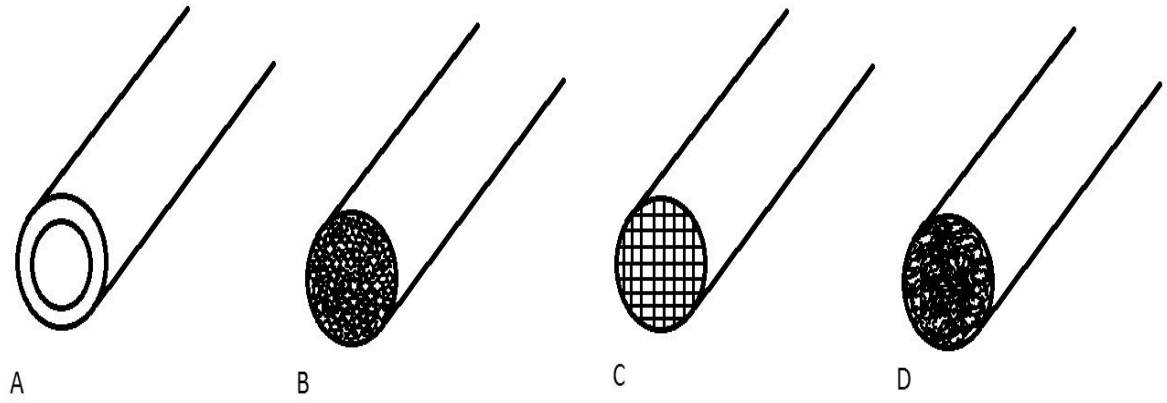

Figure 11. Different types of IT-SPME. A: wall coated, B: particle packed, C: fiber packed and D: monolith. Figure redrawn from [62].

In IT-SPME systems, the sample is repeatedly drawn in and ejected out from the capillary until equilibrium is reached. This is called draw/eject extraction. Like in other types of SPME, non-equilibrium extraction is also possible as long as sufficient amount of analyte is extracted. This is analogous to kinetic calibration in passive SPME. It is also possible to continuously draw sample through the capillary in flow through extraction, although this method requires larger amounts of sample. Flow through extraction is rarely used in wall coated capillary IT-SPME, but is more common with the packed types, as their greater extraction efficiency reduces the amount of sample needed to pass through the IT-SPME capillary.

The extracted compounds can be desorbed by either dynamic or static desorption. In dynamic desorption, solvent is drawn through the capillary and the analytes desorb to advancing solvent

front. Static desorption can be used for compounds which are more tightly bound to the sorbent. [63] In this desorption process, the whole capillary is filled with solvent until the analytes are desorbed and then ejected into the chromatographic system. A wash step between extraction and desorption can also be employed to clean the IT-SPME capillary and flow lines. This must be done with a solvent with weak elution strength towards the analytes, as otherwise loss of analytes will result.

IT-SPME is nearly always combined with LC analysis, although some examples of other separation methods [64, 65] or even without separation exist [66]. There are also new formats of IT-SPME which are designed for easier coupling with GC analysis, such as INCAT [67] and SPDE [68].

4.1 Automation of in-tube solid phase microextraction

Modifying most LC autosamplers for on-line IT-SPME is relatively easy and has been achieved with a variety of commercial models, with the Agilent 1100 LC autosampler being perhaps the most popular base for modification. [69] This is in contrast to fiber-SPME or TFME, which require specialized equipment for effective automation with LC. An example of a fully automated draw/eject IT-SPME-LC system is shown in Figure 12.

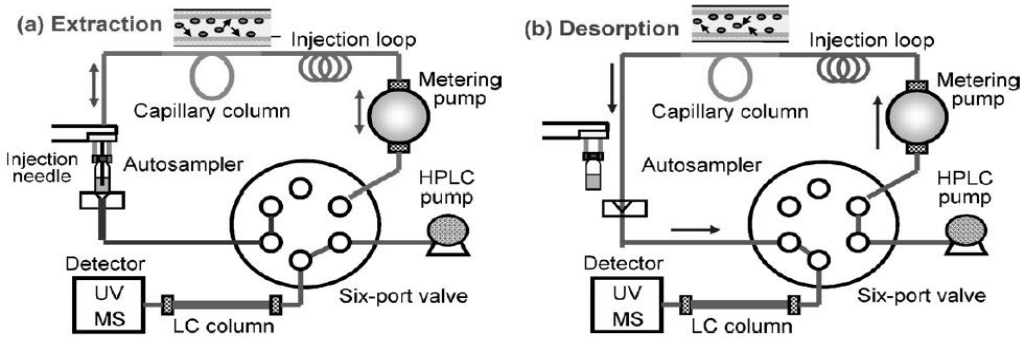

Figure 12. Draw/eject IT-SPME system. [62] Reprinted with permission from Elsevier B.V.

Because most LC pumps only pump in one direction and must be protected from contamination, a separate metering pump handles the pumping of the sample back and forth through the capillary. The metering pump itself is protected by the long injection loop before it, so that the sample never reaches the pump as it moves in the flow lines.

In static desorption, the extraction capillary is first filled with desorption solvent. After sufficient desorption time, the six-port valve is switched to inject position and the solvent filling the extraction capillary is injected into the LC column. In dynamic mode the six-port valve is switched to inject position immediately and the solvent is flushed through the extraction capillary into the LC column.

The system shown in Figure 12 does not include a wash step between the extraction and desorption.

Flow-through extraction systems are technically slightly more complicated, because sample flows in only one direction. Therefore, a separate flow line for the sample is required, as well as an outlet from which the sample can exit (Figure 13). However, it is easier to include a wash step in flow-through extraction by adding another pump for the wash solution. In injection mode the LC pump directs a flow of mobile phase through the capillary and into the column.

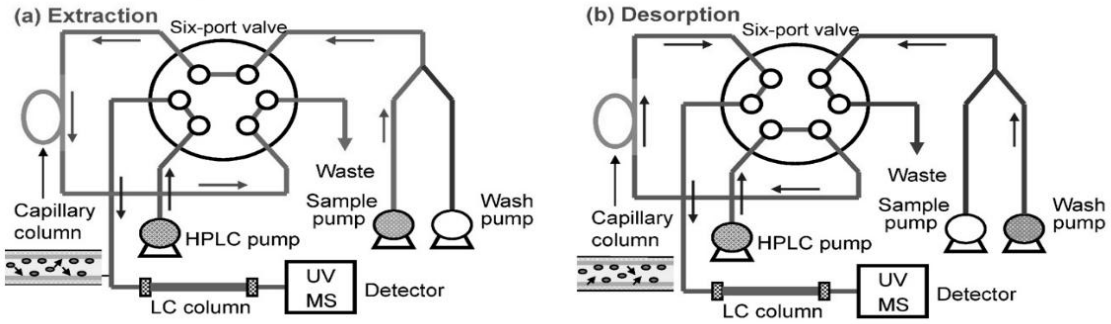

Figure 13. Flow-through IT-SPME system. [62] Reprinted with permission from Elsevier B.V.

4.2 Sample mixing in in-tube solid phase microextraction

When extraction is performed without a wash step, mixing of the remaining sample with desorption solvent can be problem. This arises mainly from the hardware and software limitations of LC autosampler systems. The exact location of the sample mixing was investigated by Raghani et al. [70] On the internal surfaces before the metering pump, mainly the extraction capillary, sample loop and associated flow lines, residual mobile phase from the previous desorption will remain. When the sample is drawn in, it contacts the residual mobile phase and mixes with it, causing cross-contamination of the mobile phase. It was also shown that the more draw/eject cycles are used, the greater the contamination will be. On the other hand, the most obvious solution to this problem, ejecting a larger volume of sample than was drawn in, may not be allowed by the LC autosampler software. In other words, only the volume that was drawn can be ejected. The problem of sample mixing was demonstrated by Yang et al. [69] by using an uncoated retention gap in place of the extracting phase, and using a wash step to flush the flow line before introducing the mobile phase (Figure 14). It is clear that sample residue remains if no wash step is used. It was also noticed that contamination was greater in a larger diameter capillary, due to higher volume of residual mobile phase.

Sample mixing is usually not a problem in flow through extraction, as the sample is only drawn in one direction. In addition, a wash step is relatively easily included in flow through extraction by for example switching the sample vial to a wash solvent vial.

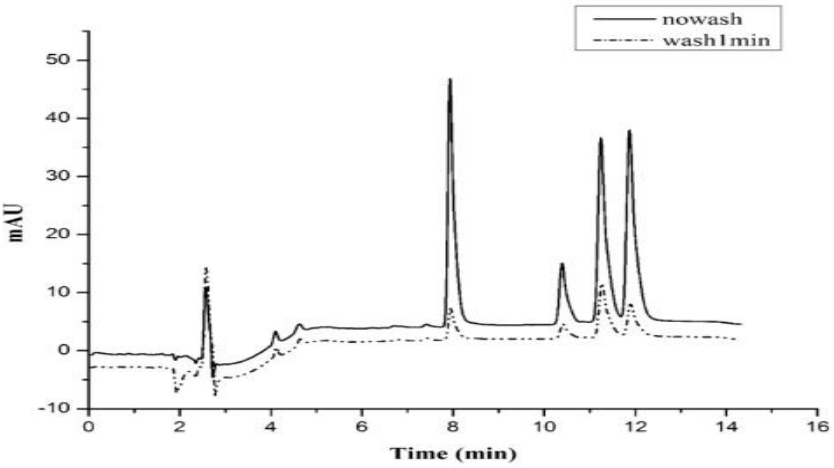

Figure 14. Extraction of PAHs from water with a 0.25 mm i.d. deactivated retention gap with and without washing step. Analysis with LC-MS. [69] Reprinted with permission from Elsevier B.V.

There have been several suggestions on how to reduce or prevent sample mixing. Wash step between extraction and desorption was already introduced by Mullet et al. in 2002. [71] This eliminates the residual mobile phase quite effectively, but may require further modifications in draw/eject extraction, because an extra flow line is required for the wash solvent. This was achieved by Mullet et al. with an additional six-port valve and software modifications. Moreover, the composition of the wash solvent should be carefully considered in order not to elute any extracted analytes. Yang et al. later suggested that the system introduced by Mullet et al. may be improved with different placement of the two six-port valves. [69]

Raghni et al. showed that an air plug drawn from the headspace of the sample vial before the extraction can help to prevent the sample coming into contact with the mobile phase. [70] This reduces sample mixing, especially in the parts after the extraction capillary (i.e. sample loop), but does not prevent it in the capillary itself. The strength of the air plug method is that it does not require any hardware or software modification and can easily be incorporated into an automated analysis by drawing in the headspace gas from the sample vial or from an empty vial for those systems which do not allow for adjustment of the needle height.

4.3 In-tube solid phase microextraction combined with other separation methods

Although in a great majority of studies with IT-SPME liquid chromatography has been the analysis method of choice, there are a handful of examples of other separation methods being used as well.

Gas chromatography has been utilized in several studies. [64, 72-75] Most GC systems cannot handle water or large amounts of any solvents. In addition, injection of solvents should be rapid, as slow vaporization in the injection port liner can lead to distorted peak shapes in the chromatogram in splitless injection mode. Therefore, there are several problems that need to be solved before IT-SPME can be used in combination with GC: with aqueous samples water must be removed from the extraction capillary and flow lines and the IT-SPME system must be capable of desorbing the extracted analytes in a small volume of solvent and also inject it quickly. Due to this, some studies have used off-line GC analysis, in which the IT-SPME system is not directly connected to the GC. [73-75] Instead, the IT-SPME desorption solvent is collected, from which a part is drawn into a syringe and injected to GC. This is similar to approaches with solvent desorption in TFME-GC.

In the automatic systems that have been introduced, at least to my knowledge, desorption problems were solved by using thermal desorption instead of solvent desorption. [64, 72] An automated on-line IT-SPME-GC system used by Globig et al. is shown in Figure 15. [64] It is controlled with two three-port valves. The extraction mode works similarly to regular flow-through IT-SPME. After the extraction, the capillary and flow lines were cleaned with helium flow in ambient temperature. In desorption mode, the capillary and valves were heated and a flow of helium was directed to the GC injector. Heating released the analytes from the sorbent, and they were flushed to the GC column by the helium flow.

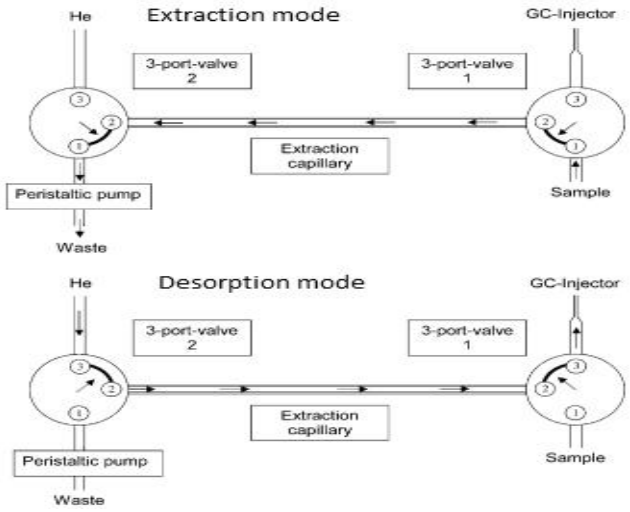

Figure 15. Automated on-ine IT-SPME-GC system in extraction mode (above) and in desorption mode (below). [64] Reprinted with permission from Springer Publishing.

In capillary electrophoresis (CE), like in LC, the sample is usually in liquid phase, which makes desorption easier, but it has only rarely been used to analyze compounds extracted with IT-SPME. Ionic compounds are usually difficult to extract with SPME, which may be the reason why CE is not often utilized. Completely automated IT-SPME-CE system is also technically difficult to build, which is a likely reason for most of the studies utilizing off-line coupling of IT-SPME and CE.

The off-line coupling is quite similar with GC: extraction is performed with a dedicated IT-SPME system and desorption eluent is collected into a vial, after which it may be pre-treated further and introduced to a separate CE system. [76-78] There are not many examples of automated IT-SPME-CE systems, but at least two have used a cross-shaped connection in combining the IT-SPME and CE capillaries. [65, 79] An example by Jinno et al. [65] is shown in Figure 16. The problem with this configuration is that the gap between the two separation capillaries has to be as small as possible in order to not to disturb the electroosmotic flow. On the other hand, this reduces the volume of the desorption solvent. Jinno et al. for example, used only about 2 µL of desorption solvent. Small desorption solvent volume may cause incomplete desoption problems.

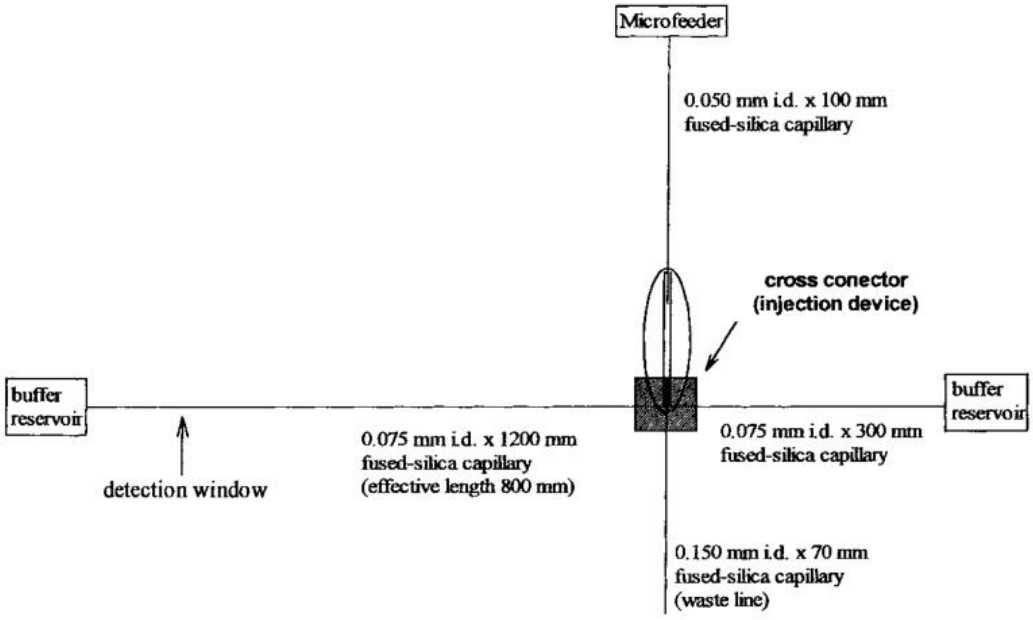

Figure 16. In-tube solid phase microextraction and capillary electrophoresis connection. [65] Reprinted with permission from John Wiley and Sons.

Similarly as with TFME, there have also been studies where separation has not been used, although examples of it appear to be rare. In one early example Mester et al. used IT-SPME to extract organic lead compounds and then directly fed the desorption solvent into an electrospray MS. [66] On the other hand, Kong et al. used a fiber packed tube to extract ionic lead and cadmium for anionic

stripping voltammetry. [80] By using a polypropylene fibers grafted with acetylic acid groups exhaustive extraction was achieved when the pH of the sample was adjusted to between 3.5 and 6.5.

There are some examples of IT-SPME being directly coupled with ICP-MS, such as those by Li et al. [81] and Zheng et al. [82] Similar to previous examples, as elemental composition was the main interest, no chromatographic separation was necessary. It seems possible to combine IT-SPME with other liquid-fed analytical methods such as atomic absorption spectrometry or spectrophotometry, although no examples exist to my knowledge. The main reason for unpopularity may be that most elemental analysis methods are sensitive and selective enough to make preconcentration and extraction methods such as IT-SPME unnecessary. As an SPME method, IT-SPME is likewise unsuitable for removing interfering compounds, as only a part of them can be extracted. Moreover, few IT-SPME sorbents are effective for extracting ionic compounds.

4.4 More precise control of in-tube solid phase microextraction

In the last few years several research groups have sought for more precise control of the extraction and desorption process in IT-SPME than can be achieved with just changing the solvents or sorbents used. In this section, a short review into methods which have been studied is presented. It should be noted that as these control methods are relatively new and none of them have been widely adopted.

4.4.1 Temperature control

Yang et al. studied a custom built IT-SPME system where the temperature of the extraction capillary could be adjusted rapidly. [83] It was discovered that all the analytes had the highest extraction efficiency at the lowest temperature setting of 10 °C, as can be seen from Figure 17. However, the relative difference between the highest and lowest temperature varied between the analytes. Yang et al. theorized that adsorption to the sorbent is an exothermic process, which according to the Van't Hoff equation will be influenced more by external temperature in compounds that have larger molecular weight, and hence larger enthalpies. This was confirmed by the results, as Angiotensin I (MW 1296) had a higher difference in extracted amounts compared to propranolol (MW 259) and ranitidine (MW 314).

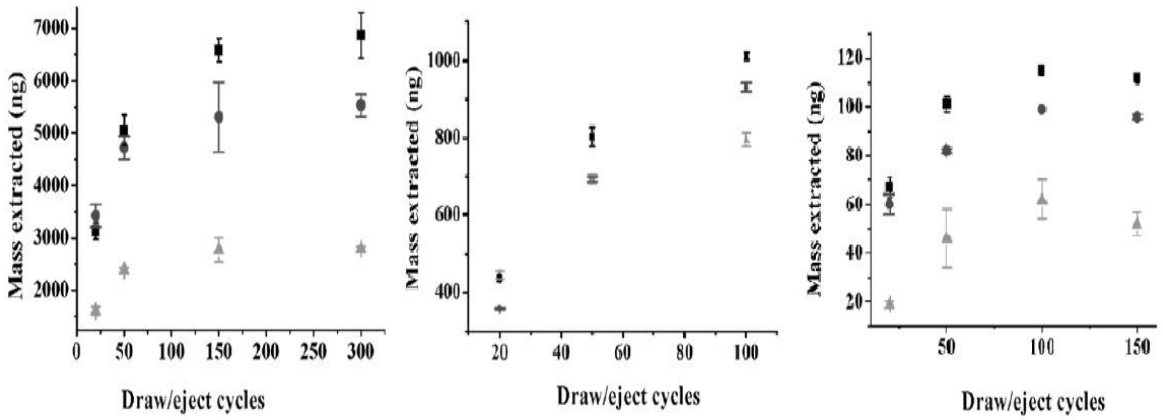

Figure 17. Effect of the temperature on the amount of analyte extracted by draw/eject extraction. Triangle: 60 °C, circle: 30 °C and square 10 °C. Analytes from left to right: angiotensin I, propranolol and ranitidine. All analytes spiked in water, analysis by LC-UV. Error bars represent standard deviation of three extractions. [83] Reprinted with permission from John Wiley and Sons.

The effect of extraction capillary temperature on the in desorption was also studied by Yang et al. [83] As expected, higher temperatures led to faster desorption and narrower peaks on the chromatogram. In this case, the greatest improvements were seen with compounds that had the highest partition coefficient.

Yu et al. took the concept of temperature control one step further by utilizing a material which changes its properties as a function of temperature. [84] However, the extraction capillary manufacturing process was quite complicated. Firstly, silica nanoparticles were immobilized on the inner surface of a fused silica capillary. After this, 3-(triethoxysilyl) propyl methacrylate was bonded, which was then modified with poly(N-isopropylacrylamide), which can alter its hydrophobic and hydrophilic properties as temperature changes. This capillary was then used in the extraction of diethylstilbestrol from water and milk. A definite response with temperature was observed (Figure 18).

Unfortunately, the extraction efficiency increased at higher temperatures. The results from Yang et al. [83] showed the opposite, with increasing efficiency in lower temperature. Therefore, poly(N-isopropylacrylamide) may not be an optimal sorbent for temperature controlled IT-SPME.

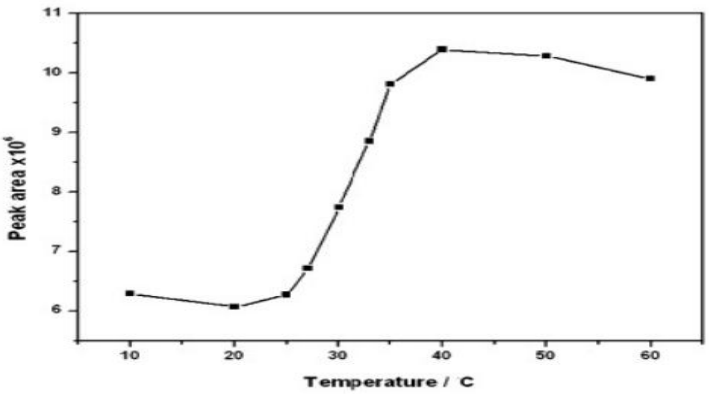

Figure 18. UV detector response as a function of temperature in the IT-SPME extraction of diethylstilbestrol from water with a poly(N-isopropylacrylamide)-modified silica capillary. Diethylstilbestrol was spiked with a concentration of 0.2 µg/L. [84] Reprinted with permission from Elsevier B.V.

An attempt with a similar concept was made by Zheng et al. with packed type of IT-SPME by using poly(N-isopropylacrylamide). [82] In this case varying temperatures between 10 and 50 °C was found to have no correlation with extraction efficiency. Zheng et al. did not attempt to find a reason why change of extraction temperature did not have any effect on the extraction. However, the material proved to be a good sorbent for ionic cobalt, nickel and cadmium.

Although controlling the temperature in IT-SPME has not yet been studied thoroughly, according to initial results it may be possible to increase extraction efficiency of especially larger compounds by just cooling the extraction capillary. For compounds with molecular weights of about 300 or less, cooling the capillary does not substantially increase the extraction efficiency. In addition, the freezing point of the sample matrix places limitations on the magnitude of the cooling. Potentially even higher efficiencies and control over the extraction can be achieved by combination with sorbent materials that change their properties according to temperature.

4.4.2 Magnetic control

The idea of using magnetic microfluidic properties of certain materials in IT-SPME was first introduced by Moliner-Martínez. [85] A homogenous 10 µm layer of Fe_3O_4 nanoparticles was deposited on the inner walls of a fused silica capillary, which was then used as the extraction capillary. When a weak magnetic field is applied from coils around the capillary, the particles become magnetized. They become demagnetized when the magnetic field is turned off. The effect of the magnetic field in the flow-through IT-SPME extraction of various compounds from water can be seen in Figure 19. The extraction efficiencies can be compared with a normal wall coated IT-SPME system, which has a typical extraction efficiency of 10-30%. [85]

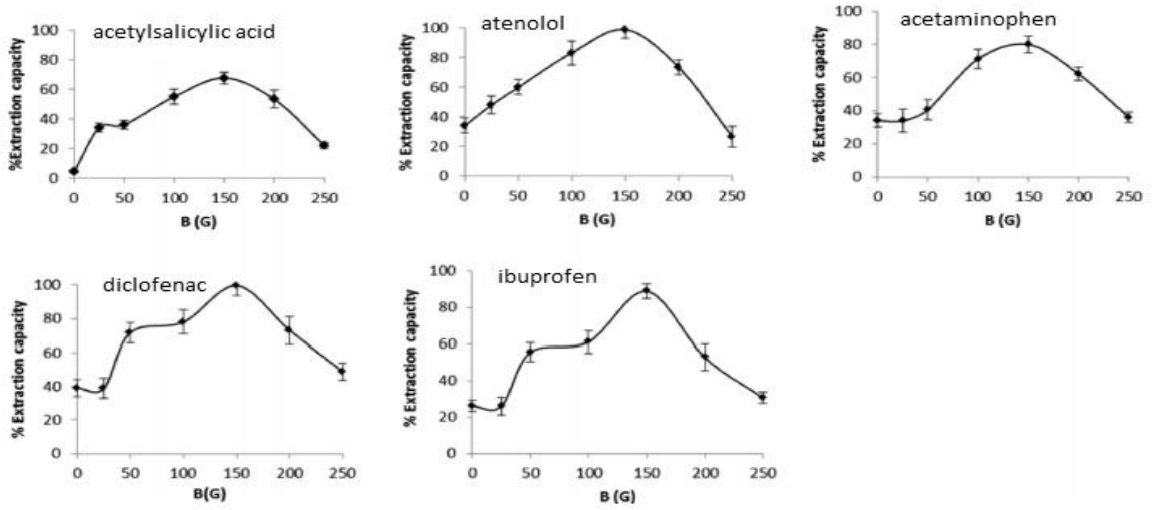

Figure 19. Extraction efficiency as a function of magnetic field strength. Each analyte with a concentreation of 50 µL/L, injection 200 µL. Analysis with LC-UV. [85] Reprinted with permission from American Chemical Society.

All the analytes were diamagnetic, meaning that they are repelled by the magnetic field induced on the Fe_3O_4 nanoparticles. The analytes were concentrated in areas where the magnetic field was the weakest. This effect was apparently very strong, as Moliner-Martínez et al. were able to reach high efficiency or even exhaustive extraction on some of the analytes. Desorption was minimal even when mobile phase was directed through the extraction capillary while it was magnetized. In desorption, the polarity of the magnetic field was reversed to induce the release of the analytes. The same group also has published an application of magnetic IT-SPME for the determination of organophosphorus compounds from wastewater with similarly high extraction efficiencies. [86] As magnetic IT-SPME is a recently introduced technique, there is still lack of research in its possibilities and limitations.

4.4.3 Electrochemical control

The idea of using polypyrrole (PPy) in SPME is not new, and it was already introduced for fiber-SPME [87] and IT-SPME [88] more than a decade ago. However, research into PPy's electrochemical properties in IT-SPME was recently introduced by Ahmadi et al. [89]

The system for electrochemically controlled IT-SPME is nearly the same as in normal IT-SPME. The only difference is that a constant electrical potential is applied to the extraction capillary, which causes PPy to become positively or negatively charged. The charged PPy will naturally attract the ions of the opposite charge passing through the capillary. In order for electrochemical control work efficiently, the sample matrix must be sufficiently conductive. Adding ionic components as modifiers may therefore increase extraction efficiency. However, at too high concentration non-

analyte ions will compete with analytes and reduce the amount of analytes that can bind to the sorbent. This also includes very high or low pH values. NaCl was recommended as the best modifier due to the small size of its ions. [90]

Along with factors that affect the extraction efficiency in normal IT-SPME, electrochemical IT-SPME introduces additional parameters that must be optimized. Using Plackett-Burman experimental design, Ahmadi et al. studied the different parameters which affect the extraction in flow-through extraction. [91] The factors were (from largest effect to the smallest): potential of the extraction, pH of the sample, extraction time, desorption time and flow rate of the extraction.

Electrochemical IT-SPME is an attractive choice for ionic analytes, which are problematic to extract with more traditional sorbent materials. The degree of ionization for many compounds can be easily controlled by changing the pH of the sample solution, although extremely low or high concentrations may disrupt the analyte extraction process.

4.5 Derivatization and in-tube solid phase microextraction

Although general trends in analytical chemistry are in favor of reducing or elimintating derivatization wherever possible, for certain analytes it is still necessary. There are many examples of derivatization with IT-SPME, although in most cases the derivatization was done in sample first, after which the derivatives were extracted with IT-SPME. [92-94] However, this type of derivatization is difficult to automate as it requires manipulation of the sample. On-sorbent derivatization is easier to automate, as was shown by Prieto-Blanco et al. [95] In this approach, first a solution containing derivatization reagent (9-fluorenmethylene chloride) was extracted by IT-SPME. After this, the actual sample containing the analyte was simultaneously extracted and derivatized. This remains the only fully on-line example of derivatization and IT-SPME to my knowledge. A reason for this may be that in many cases on-sorbent derivatization does not have satisfactory performance in IT-SPME. In a different study Prieto-Blanco et al. compated several methods of derivatization in IT-SPME. [96] They noted that on-sorbent derivatization had much reduced sensitivity compared to in-sample derivatization before extraction.

4.6 Wall coated capillaries for in-tube solid phase microextraction

Wall coated capillaries have been used since the first studies with IT-SPME and they have the largest body of research behind them. Their definite strength is that there is already a large variety of GC capillary commercially available for both polar and nonpolar analytes. It is also possible to coat fused silica capillaries in lab either with GC capillary phase or with custom phase. [97]

It should be noted that although WCOT GC columns were used in many early studies, in recent years when commercial capillary has been the choice of extraction capillary in IT-SPME, a PLOT column has often been used instead. PLOT columns have a larger adsorption surface area and higher volume of sorbent phase, which translates to higher efficiency and larger capacity. However, as with other types of SPME, in accordance with Equation 6, due to the larger sorbent thickness it may take longer to establish equilibrium. [98] Kataoka et al. have compared PLOT columns as extraction capillaries to WCOT columns in several studies. [99-102] The PLOT capillaries performed better than the WCOT capillaries for example in the extraction of ochratoxins from nuts, [99] steroidal hormones from saliva [100, 101] and heterocyclic amines from liquid extracted hair samples. [102] An example from one of their studies is shown in Figure 20, from which can be seen that Supel-Q divinylbenzene PLOT column has relatively high extraction efficiency with nearly all the analytes. However, although PLOT columns usually have better extraction efficiency, the optimal sorbent material is still dictated by the analytes. For example, in the extraction of ochratoxins from nuts, it was Carboxen 1010 PLOT which had better response. [99]

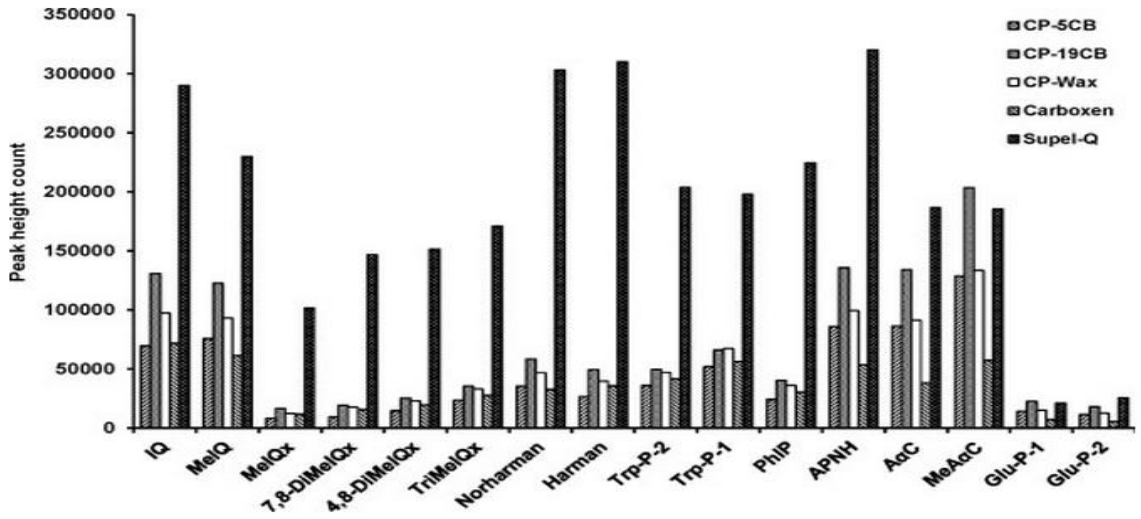

Figure 20. Comparison of several commercial GC columns as extraction capillary for in-tube solid phase microextraction. The analytes were heteroaromatic amines in water, which were analyzed with LC-MS/MS. GC capillary column stationary phases were 5 µm 100% PDMS (CP-5CB), 1.2 µm 14% cyanopropyl phenyl methylsilicone (CP-19CB), 1.2 µm 100% polyethylene glycol (CP-Wax), 15 µm Carboxen 1010 PLOT (Carboxen) and 17 µm 100% divinylbenzene PLOT (Supel-Q). [102] Reprinted with permission from Elsevier B.V.

GC capillary columns are designed for different types of conditions than in what they are used in IT-SPME. Unlike in GC, a large volume of liquid sample is directly in contact with the stationary phase in practically all IT-SPME applications. The sample may damage the stationary phase film. Moreover, commercial GC columns are designed for separation of many types of analytes, so they

rarely have selective affinity. Instead, they have varying degrees of sorptive properties towards many compounds.

Due to the general affinities and limited volumes of GC column stationary phases, some researchers have made studies with custom made sorbents. [87, 103] Unlike the stationary phases of GC capillary columns, IT-SPME extraction capillary sorbents usually do not need to be resilient to high temperatures. This makes it is possible to use materials with poor temperature resistance. However, new sorbent materials are more common with the packed types of IT-SPME, as immobilizing the sorbent on the capillary walls can be problematic.

PPy has already been noted for its electrochemical properties. It can also act as a sorbent without using this property, although with less control over the extraction process. It has many attractive properties for extraction: as an aromatic compound it interacts with other aromatics, and also it is polar, interacting with polar compounds. Moreover, it is a positively charged weak acid, so it can have strong interactions with anionic analytes. Changing the pH of the solution can affect the charge of the analytes and PPy, which gives a degree of control over the extraction process. [98] As noted earlier, the amount of the positive charges on the polymer can also be changed much more quickly via application of an electric potential. [90] Finally, PPy has a porous structure when coated on the inside walls of an IT-SPME capillary, which results in high surface area. IT-SPME with wall coated PPy sorbent was subject to a number of studies in the early 2000's. [88] It was found to be superior to many commercial GC capillaries in the extraction of nitrosamines from cell cultures [104] and polar pesticides from wine samples. [105] However, since then there was little research activity for it as a sorbent for IT-SPME until the introduction of electrochemically controlled IT-SPME in 2014. [90]

In order to add more selectivity to extractions, some researches have attempted restrict access to sorbent to only analytes of interest. One such way is to utilize immunoaffinity sorbents, which have a very high degree of selectivity. Ideally, one type of sorbent can only bind one type of molecule, although in practice nonspecific binding can also occur. A highly selective sorbent would allow extraction from even complicated matrices such as blood with little preparation. Immunosorbents have been used in fiber-SPME, and have also been applied to IT-SPME by immobilizing them on the inside walls of silica capillaries. [63, 103] For example, Queiroz et al. used fluoxetine antibody sorbent to extract fluoxetine from blood serum, although nonspecific binding of norfluoxetine was also observed. [103] Chaves et al. immobilized anti-interferon α2a antibody onto silica capillary and extracted interferon α2a from blood plasma. [63] However, there are some problems in using immunoaffinity sorbent. Obtaining sufficient quantities of pure biomolecules to be used as an

extraction phase can sometimes be difficult. Furthermore, as the sorbent is composed of proteins, it can quickly lose effectiveness, sometimes permanently, in non-optimal conditions due to the denaturation of the biomolecules. Finally, due to the relatively strong forces involved in immunobinding, optimization of the desorption process is very important.

4.7 Particle packed in-tube solid phase microextraction

IT-SPME with extraction capillaries packed with sorbent particles has been quite rare, as frits are required to hold the particles in place. Mostly it has been applied for specialty materials which are difficult to immobilize on the walls of a capillary or on fibers. One such example is by Mullet et al. who used an alkyl-diol-silica sorbent as a restricted access material (RAM) to extract benzodiazepines directly from human blood serum. [71] This two layered RAM prevents the entry of molecules over certain size to the actual sorbent phase, reducing the problems arising from irreversible adsorption of large biomolecules to the sorbent. Therefore, RAMs can be thought as a kind of a filter. The particles were simply packed into a piece of PEEK tubing, and both ends fitted with frits. This tube then served as the extraction capillary. The alkyl-diol-silica capillary was tested up to 100 extractions with minimal adsorption of proteins. Later, Chaves et al. used a different type of RAM (bovine serum albumin coated silica) packed in a similar manner to extract interferon α from a direct injection of human blood plasma. [106] It was noted that the material could exclude more than 90% of unwanted proteins and required only one draw/eject cycle to reach a limit of quantification of 0.06 mIU/mL.

As immunoaffinity sorbents are mainly restricted to biologically active analytes and RAMs have only limited selectivity, some researches have worked with molecularly imprinted polymers (MIPs) instead. These materials are polymerized around an analyte (a template), which is then washed off. The cavities left by the analytes can be then used to selectively extract the same compound. Similarly to immunoaffinity sorbents, MIP sorbents are typically selective, but not specific, as compounds structurally similar to the template may also bind to the cavities. To my knowledge, MIPs have not been utilized in wall coated IT-SPME, but have been packed into PEEK tubes in particle form similarly to the RAM materials. [107]

For example, Queiroz et al. utilized a sol-gel generated MIP in the extraction of interferon α2a from blood plasma. [108] Compared to the immunoaffinity sorbent by the same authors, the MIP sorbent had less selectivity, but could be produced from cheaper materials with more reproducibility. On the other hand, it had more selectivity than the RAM sorbent. Mullet et al. noted in a different study that although MIP had selectivity for the analyte (propranolol), non-specific binding of other beta blockers was observed as well. [107]

4.8 Fiber packed in-tube solid phase microextraction

The concept of inserting a wire into an IT-SPME extraction capillary was introduced by Saito et al. in 2000. [109] In the first study, a stainless steel wire was inserted into the middle of a capillary to reduce its internal volume. As the interaction between the wall coated sorbent and the analytes immediately next to it are the main cause of extraction in IT-SPME, by removing the extra volume from the middle of the capillary where no interaction between analytes and sorbent exist, higher extraction efficiencies are possible. An added benefit is that due to the reduced volume of the extraction capillary, less desorption solvent is required. Soon afterwards, Saito et al. replaced the single thick stainless steel wire with multiple fibrous polymers with sorbent capability of their own, thus introducing the current fiber packed IT-SPME format (Figure 21). [110] It was also noted that the smaller the free volume in the extraction capillary, the greater the extraction efficiency, as more analyte-sorbent interactions will occur. This was shown by Jinno et al. in their study where different packing densities were compared (Figure 22). [65] The capillary with higher packing density has considerably better extraction efficiency in the same conditions.

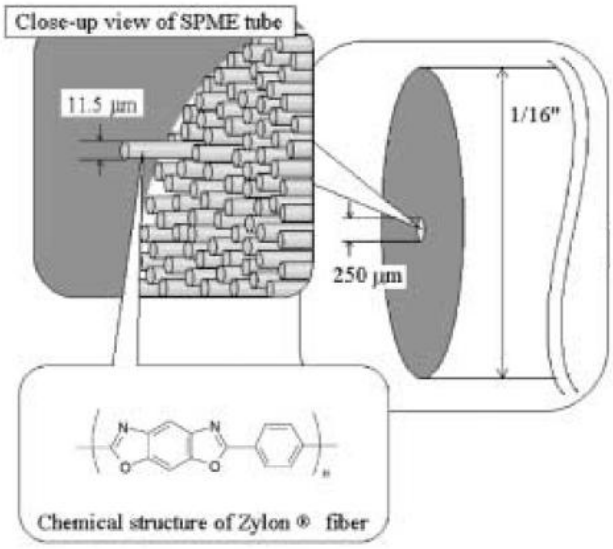

Figure 21. First fiber packed IT-SPME extraction capillary. Packing was made of 11.5 µm poly(p-phenylene-2,6-benzobisoxazole), trade name Zylon, polymer fibers. [110] Reprinted with permission from Springer Publishing.

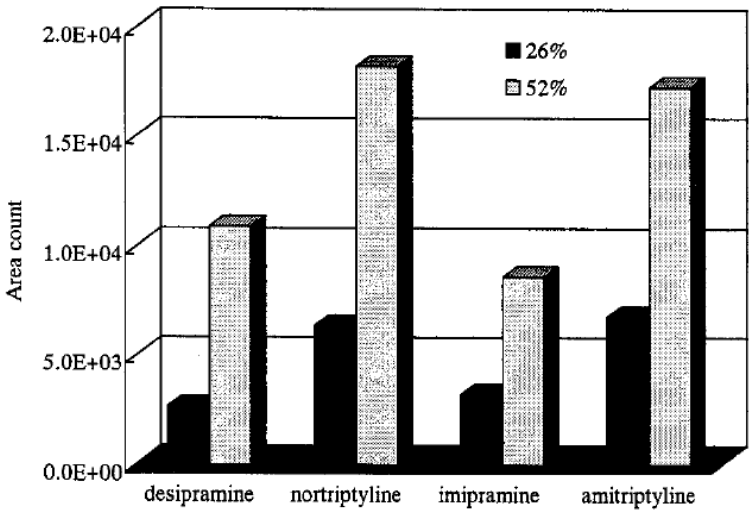

Figure 22. Effect of packing density of poly(p-phenylene-2,6-benzobisoxazole) fibers on the extraction efficiency of antidepressant drugs from urine. 26 % packed DB-5 GC column and 52 % DB-5 column were used as the extraction capillaries. Each analyte with concentration of 0.5 µg/mL, analysis with CE-UV. [65] Reprinted with permission from John Wiley and Sons.

The extraction efficiencies with fiber packed extraction capillaries can be very high. Saito et al. reported 90% extraction efficiency for phthalates from water with a poly(p-phenylene-2,6-benzobisoxazole) fiber packed PEEK tube. [111] They called the technique miniaturized SPE instead IT-SPME due to the nearly exhaustive extraction. This is an important distinction from IT-SPME noted by other authors as well. [112, 113] In SPME sample volume is not of great concern, but in exhaustive extraction control of it is extremely important in order to avoid breakthrough. However, it is often unclearly reported whether extraction mode is based on equilibrium or if it is exhaustive, as extraction efficiency or breakthrough have not been investigated. For example, Hu et al. reported enrichment factors, meaning peak areas compared to those with direct liquid injection without extraction, between 69 and 136 for the analytes. [114] Liu et al. achieved even higher enrichment, reporting an 88 to 307 fold increase in peak areas. [115] This high enrichment would lead one to suspect that extraction is exhaustive, but both authors called the extraction technique IT-SPME and did not investigate if exhaustive extraction had been achieved.

Fiber-packed IT-SPME extraction capillaries are relatively easy to make. Similarly to particle packed type, most authors have used a PEEK tube packed with the fibers. Only a few sorbent types have been studied, such as poly(p-phenylene-2,6-benzobisoxazole), MIP coated silica and carbon nanotubes. [110, 114, 115]

4.9 Monolith in-tube solid phase microextraction

The creation of monoliths inside silica capillaries has been studied extensively, and they have also been adapted for IT-SPME. The properties of the monolith are controlled by the properties of the monomers used to polymerize it. As monoliths are synthesized directly inside the capillary, there is no need for any kind of packing process, with an added benefit that monoliths do not require frits to hold them in place. Similarly to other packed extraction capillaries, monolith IT-SPME has higher extraction efficiency that the wall coated type due to increased interaction by analytes with the sorbent. In some cases even exhaustive or near exhaustive extraction has been reported. [116-119] Like with fiber packed IT-SPME, many authors only compare detector response when optimizing an extraction method, and do not report extraction efficiencies or if breakthrough has been observed. This makes it difficult to know whether equilibrium or exhaustive extraction is in question.

Due to the possibility of varying the properties of monoliths, many types of monoliths are custom made to extract certain types of analytes. The most common type of monolith encountered in literature is made of poly(methacrylic acid–ethylene glycol dimethacrylate). It has been used in the extraction of a number of different analytes from various sample matrices, for example, drugs from blood serum and urine [120, 121], antibacterial residues in milk [122], tetracycline antibiotics in fish muscle [123] and telmisartan from rat tissue [124]. Other types of monoliths have also been studied, and some examples of these are listed in Table 3. As can be seen, many of these incorporate inorganic or ionic compounds in order to increase extraction efficiency of polar or ionic compounds. It is also possible synthesize MIP monoliths, which offers a more effective way to introduce MIPs as extraction phases for IT-SPME.

Table 3. Monolith types used in IT-SPME.

Monolith material	Analytes	Matrix	Reference
C18 bonded silica	Alkylphenolic pesticides	Water	[119]
Poly(acrylamide-vinylpyridine-N,N′-methylene bisacrylamide)	Non-steroidal anti-inflammatory drugs, phenols, non-peptide angiotensin II receptor antagonists and endocrine disrupting chemicals	Water	[125]
Glycoprotein MIP	Glycoproteins	Diluted egg white	[126]

Table 3 continues.

Monolith material	Analytes	Matrix	Reference
SiO_2/TiO_2 composite	Phosphopeptides	Digested and diluted egg white	[127]
Lysozyme MIP	Lysozyme	Diluted egg white, blood serum	[128]
Hybrid organic–inorganic silica with cyanoethyl functional groups	Antidepressants	Urine, blood plasma	[129]
Carbon nanotubes incorporated polymer	Triazine herbicides	Water	[130]
Ionic liquid-modified organic polymer	Aromatic carboxylic acids	Water	[116]

The most problematic part of monolithic extraction capillaries is their production. The pore size needs to be carefully controlled in order to minimize backpressure, but also to ensure sufficient extraction efficiency. [114] Capillary to capillary repeatability has rarely been tested, but Lin et al. reported a relative standard deviation in peak areas of less than 4% between five capillaries for the same extraction. [128]

4.10 Array in-tube solid phase microextraction

Removing the on-line coupling of IT-SPME to LC enables using many IT-SPME capillaries at the same time as an array. One example of this is the array capillary in-tube solid phase microextraction developed by Yan et al. that was used for analysis of PAHs, chlorobenzenes and nitrochlorobenzenes from water. [131, 132] The array capillary IT-SPME consists of a GC liner, which is packed with glass capillaries (Figure 23). Both the inside and the outside walls of the glass capillaries were covered with sol-gel PDMS coating, which serves as the extraction phase. The permeability of the array capillary device is large enough that water sample flows through only assisted by gravity. Since the device built in a GC liner insert, a homemade thermal desorption unit was used to desorb the analytes, and the analytes were sufficiently volatile to be focused at the front of the column. It should be noted that the array capillary was centrifuged before desorption to prevent water from entering the GC column. With this simple device, LODs of around 1 ng/L were reached for PAHs by letting 350 mL of water to flow through the device for just 2 minutes.

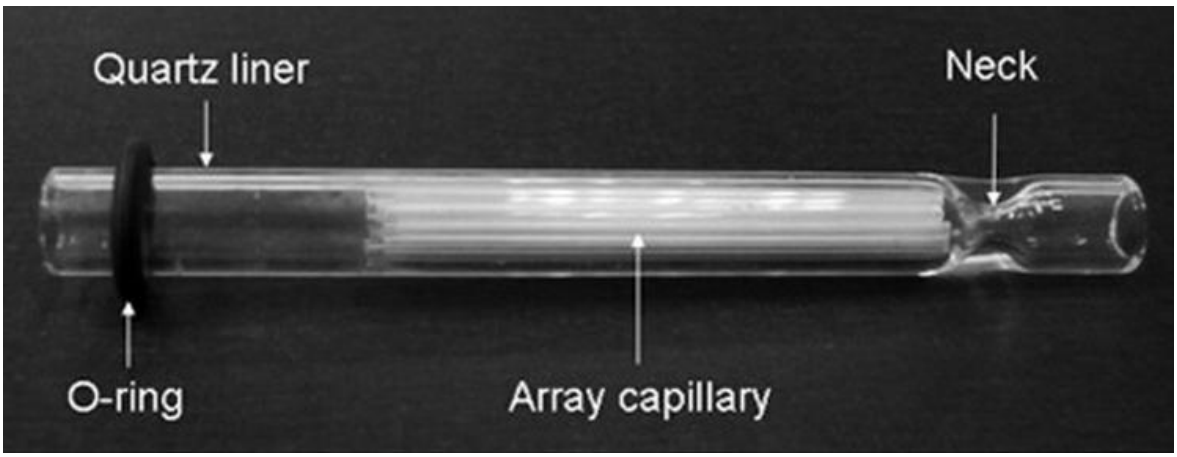

Figure 23. Array capillary in-tube solid phase microextraction device. It is composed of a 4 mm internal diameter GC liner insert packed with 19 0.5 mm internal diameter glass capillaries coated inside and outside with PDMS. [131] Reprinted with permission from Elsevier B.V.

5. In-tube capillary trap and solid phase dynamic extraction

In-tube capillary trap and solid phase dynamic extraction can be thought as further developments of IT-SPME. They are needle-like in tube devices more suitable to GC analysis. In-tube capillary adsorption trap (INCAT) is a device where an extraction capillary is placed inside a steel needle. This makes it more resistant to handling and allows for easy integration with GC. It is also possible to coat the inside walls of a needle directly with the sorbent and dispense with the capillary altogether. These types of extraction devices are usually called solid-phase dynamic extraction (SPDE) devices, but they are also sometimes referred to as INCAT devices, depending on the article. INCAT was introduced by McComb et al. in 1997 [67] and SPDE by Lipinski in 2001 [68]. Soon after its introduction SPDE was commercialized as “magic needle” SPDE device by Chromtech. In contrast to IT-SPME, INCAT and SPDE have been used mainly to extract compounds from the gas phase. See Figure 24 for a graphical representation of these techinques. There is confusion on how to classify the multitude of new techniques that have appeared after the success of fiber-SPME, including INCAT and SPDE. For example, in some studies adsorbent-packed metal needle was referred as INCAT device, [133, 134] although these types of devices are usually referred as needle traps or needle extractors. Similarly, there is significant overlap between the terms INCAT and SPDE. [135] For clarity, the term INCAT/SPDE will be used to refer to the sorbent coated needle, and INCAT for the silica capillary in needle type.

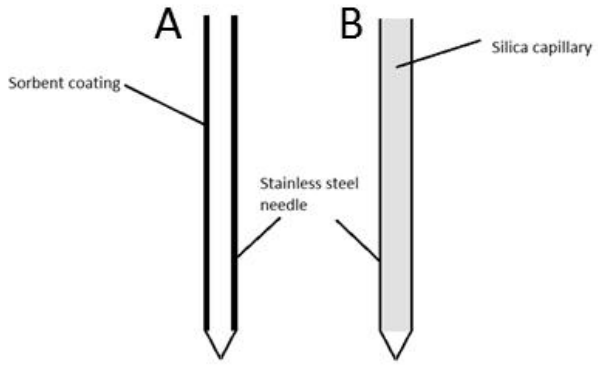

Figure 24. The two types of INCAT and SPDE. A: INCAT B: INCAT/SPDE.

Compared to fiber-SPME, INCAT/SPDE has larger extraction phase volume and is more durable due to its metal needle construction. On-line combination with GC is much simpler that with IT-SPME or TFME, as INCAT/SPDE is outwardly similar to normal GC injection syringes.

5.1 In-tube capillary trap by using a gas chromatography capillary

In the first study introducing INCAT, the device was defined as a hollow needle with a piece of capillary inside it or with an internal coating of carbon. [67] During the following years, research has focused on the latter type, where the sorbent coating is on the inside of a needle. This became to be known as SPDE after Lipisnki introduced non-carbon coatings. [68] INCAT with a GC capillary seems obsolete in practice, and there has not been any research on GC capillary in tube SPME devices to my knowledge after the late 1990's. Other techniques, such as open tubular trapping [136] and the recently introduced iterative trapping [137] do utilize GC capillaries for extraction of volatile compounds. However, they are generally exhaustive extraction methods, not SPME.

There is no clear reason why INCAT devices with a GC capillary inside are so unpopular. The main reason may be that the capillary on which the stationary phase is bound is unnecessary, and causes additional problems. For example, it may be difficult to match the diameter of the capillaries with needles. Too large a capillary does not fit and too small leaves a large dead volume inside the needle for the sample to bypass. It can also be problematic to attach the capillary in the needle. There is a large body of research on modifying the interior of silica capillaries, but it seems that most researchers prefer to modify the inside of metal needles rather than to modify a silica capillary and place it inside a needle.

5.2 In-tube capillary trap/solid phase dynamic extraction using an internally coated metal needle

The principle of the operation of INCAT/SPDE is quite similar to IT-SPME: either a solution or gas is drawn and ejected through the needle until equilibrium or sufficient amount of analytes have partitioned into the sorbent material. However, passive sampling of gas is also possible by simply placing the tip of the INCAT/SPDE device into the sample without any aspiration. [67, 138] The analytes will diffuse into the needle and partition into the sorbent material. Because the sorbent is enclosed in a needle instead of being fully exposed like in fiber-SPME or TFME, the rate of diffusion of analytes to the inside of the needle limits the speed of extraction. Therefore, the time required to reach equilibrium or extraction of sufficient amount of analytes can become quite long. In the great majority of studies with INCAT/SPDE active extraction has been used instead. Typical operation of an INCAT/SPDE draw/eject extraction is shown in Figure 25.

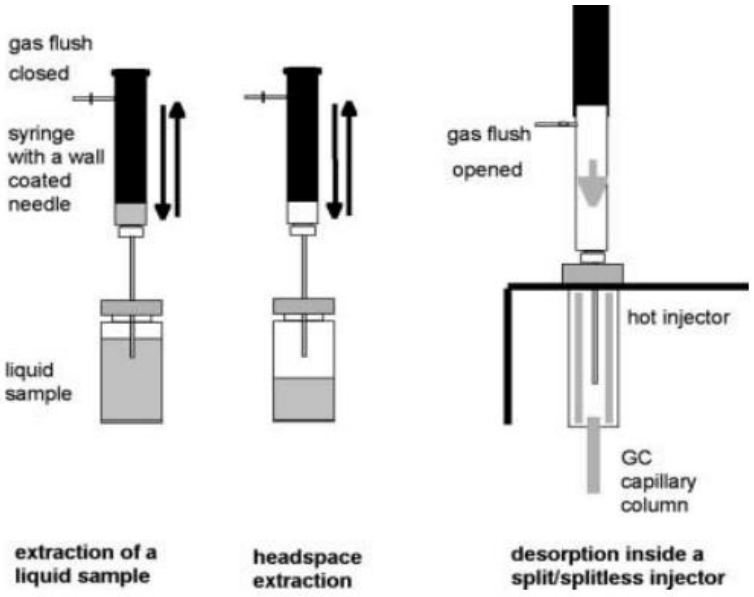

Figure 25. Direct and headspace draw/eject extraction with an INCAT/SPDE device. [68] Reprinted with permission from Springer Publishing.

Due to their needle-like construction, INCAT/SPDE needles allow for relatively easy coupling to GC, and even a fully automated device have been developed by Chromtech. As shown in Figure 25, desorption is most often achieved by placing the INCAT/SPDE device in the injection port and directing a small volume of gas through it to flush the desorbed compounds into the injection liner and to the GC column. Sample transfer to GC column has also been achieved without gas flow, although desorption times are then longer. [135, 139]

5.3 Comparison with other solid phase microextraction techniques

Unfortunately, there have not been many comparison studies where INCAT/SPDE has been compared with other extraction techniques by the same group using similar equipment. The most recent study in this topic was made by Gamero et al., where several types of SPME techniques where used to determine volatile aroma compounds from wines. [140] In this study, based on the number of aroma compounds that could be reliably detected, INCAT/SPDE was found to have only slightly better performance than headspace sampling, and lower performance than all the other tested techniques (direct SPME, headspace SPME, SBSE and monolithic material sorptive extraction). Although the authors did claim to have optimized the extraction with INCAT/SPDE, the number of draw/eject cycles was unusually low: 1 mL of headspace volume was cycled only five times, although typically more cycles are used with similar volumes. To compare a similar example, Malherbe et al. used 50 cycles of 1 mL of headspace volume to analyze fermenting grape must with the same commercial PDMS/active carbon INCAT/SPDE needle. [141] Rossbach et al. used ten draw/eject cycles, but the volume with each cycle was 2.5 mL. [142] Therefore, the reason for the poor performance of INCAT/SPDE could be the improper application of the technique.

Jochmann et al. compared the LODs of their INCAT/SPDE method using a PDMS/activated carbon needle to values found in literature for fiber-SPME with PDMS/CAR sorbent. [143] Analytes were volatile hydrocarbons in water. For dichloromethane and carbon tetrachloride INCAT/SPDE achieved LODs of two orders of magnitude smaller than SPME, but an order of magnitude higher for chloroform. For others, the LODs were roughly equivalent.

From these results, it is not clear if INCAT/SPDE has clear advantage over fiber-SPME. Most likely the effectiveness must be evaluated on an individual basis, as it depends on the sample matrix and analytes of interest.

5.4 Factors influencing extraction efficiency

The influence of various factors on the extraction efficiency of volatile d-limolene fragments in INCAT/SPDE was studied by Kamphoff. [144] Extraction was performed from the headspace of an enclosed sample vial with draw/eject extraction. Two major factors were discovered: temperature of the sample and number of draw/eject cycles. However, the flow rate of the draw and eject steps was found not have a large influence. Similar observations were also made by other groups. [145, 146] Jochmann et al. confirmed the same phenomenon for volatile compounds with low affinity for the sorbent. [143] On the other hand, for less volatile compounds with a higher affinity for the sorbent, increasing draw/eject flow rate decreased the extraction efficiency. This has been also

observed with other sorbents that have high affinity towards analytes, [139] and it agrees with Equation 10. Forcing analytes to move faster through the needle reduces their interaction with the sorbent and reduces extraction efficiency. For analytes with low affinity to the sorbent in the first place, this effect is less noticeable.

In the case of at least volatile compounds, the time that the liquid sample is allowed to equilibrate with the headspace has been reported to have only a small influence on the extraction efficiency, although this has been noted to increase in complicated matrices. [147] Kamphoff et al. noted that agitation of the sample had only a minor influence on extraction efficiency of highly volatile compounds, [144] but agitation is still commonly used with INCAT/SPDE.

5.5 Sorption/desorption model and accelerated extraction

Van Durme et al. investigated the mechanisms of INCAT/SPDE device with PDMS as the sorbent and toluene as a model compound. [146] Both computational and experimental results were reported, revealing several insights into the mechanism of INCAT/SPDE. It was found out that INCAT/SPDE is an equilibrium method and has very limited exhaustive extraction capability – breakthrough was already detected after sampling only 1.0 mL of 92 ppm_v toluene-air mixture. This was detected by injecting the gas pulled through the INCAT/SPDE needle in 0.5 mL steps. The results of a draw/eject extraction was compared with flow through extraction (Figure 26). The peak areas versus aspirated volumes obtained with GC-FID seem to deviate from each other right after breakthrough. The deviation becomes even clearer when moving on to non-exhaustive extraction. Curves combine only when equilibrium has been reached. Volumetric flow rate did not have large impact on extraction efficiency in this case, but as mentioned earlier, opposite results have also been reported. [139, 143]

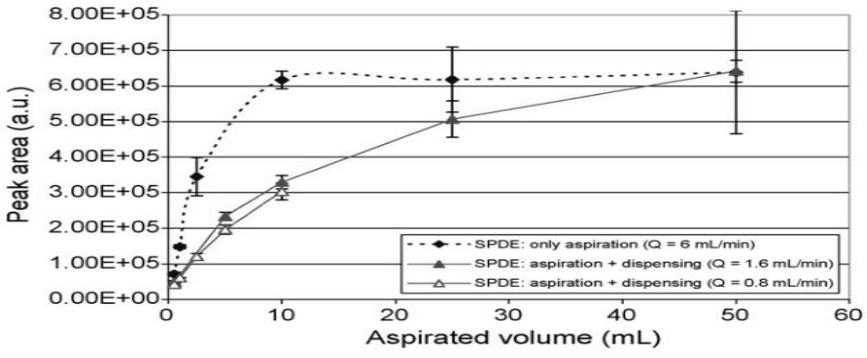

Figure 26. Comparison of peak area versus aspirated volume between draw/eject (aspiration + dispensing) and flow through (only aspiration) extractions. Draw/eject volume was 0.5 mL, Q is the volumetric flow rate, error bars represent standard deviation of three repetitions. Analysis with GC-FID. [146] Reprinted with permission from Elsevier B.V.

Based on this data, as well as computer models, a sorption/desorption theory was proprosed for INCAT/SPDE, which is shown in Figure 27 for the first draw/eject step. When the first volume of toluene-air mixture is drawn in the needle, all of the analyte will be trapped on the sorbent, and the air in the syringe is free of toluene. When this air is pushed through the needle in the ejection step, some of the extracted toluene will partition back into the toluene-free air. After breakthrough, the concentration of toluene will not be zero in the air after passing the sorbent, but it will be lower than when it was drawn from the sample. Again, partitioning losses occur from the sorbent into the air. Therefore, with each cycle in draw/eject extraction some of the analyte sorbed in the draw step will be desorbed in the ejection step, increasing the time required to enrich the sorbent with the analyte. After equilibrium has been reached, by definition, there is no net gain in draw step and no net loss in ejection step and further draw/eject cycles have no effect on the amount of analyte on the sorbent.

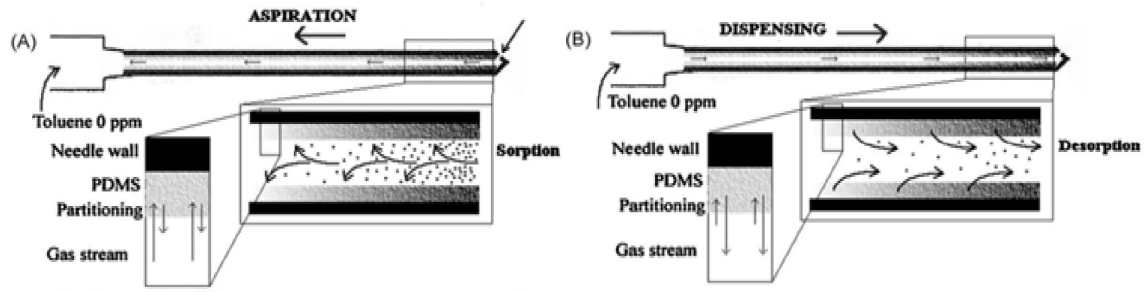

Figure 27. Sorption/desorption model of INCAT/SPDE for the first draw/eject cycles. In cycles after breakthrough cycles analyte (toluene) concentration in syringe will no longer be zero, but will be lower than in the analyte-air mixture that was drawn in. [146] Reprinted with permission from Elsevier B.V.

Van Durme et al. also presented a new accelerated extraction model, which is analogous to flow-through extraction in IT-SPME. [146] Instead of draw/eject cycles, there was only a continuous flow through the needle. Using flow-through extraction, the time required to reach equilibrium extraction of toluene from gaseous standards was just 1.7 minutes compared to 62.5 minutes with draw/eject sampling. Pokorska et al. later successfully applied flow through extraction developed to field analysis of biogenic volatile organic compounds. [148] It is noteworthy that flow-through extraction required pumping in only one direction, which makes it attractive towards field sampling systems. However, some of the same limitations that are present in flow through extraction in IT-SPME also apply to INCAT/SPDE. Namely, that much larger amount of sample is required in flow through extraction.

Despite the advantages of flow through extraction, currently draw/eject extraction is clearly preferred. The main reason for this may be that because INCAT/SPDE is mostly used for headspace

extraction in small sample vials, it follows that the amount of headspace gas in those vials is relatively limited. This may prevent flow though extraction, as INCAT/SPDE will require aspiration of around 10 mL of gas volume to reach equilibrium for even easily extracted compounds such as toluene (Figure 26). Therefore, reaching equilibrium with flow through extraction therefore may not be possible. On the other hand, equilibrium can be reached with draw/eject extraction even with small sample volumes, although it will take longer.

In order to use flow through extraction with small sample volumes, Gholivand et al. proposed circulation of the headspace gas (Figure 28). [149] The negative aspect is that this type of system increases the complexity of the device and makes automation more difficult. Gholivand et al. performed the extractions manually.

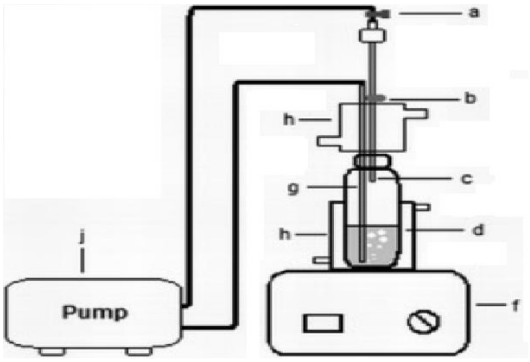

Figure 28. Extraction system used by Gholivand et al. Circulating the headspace gas allows for flow through sampling even when the headspace volume is small. a: valve, b: septum sealed the side hole, c: sorbent inside the needle, d: sample solution, f: magnetic stirrer, g: water bath, h: purging needle and j: membrane vacuum pump. [149] Reprinted with permission from John Wiley and Sons.

5.6 Direct extraction

Although INCAT/SPDE is mainly used for extraction from the gas phase, there are also examples of drawing liquid sample directly into the needle. [58, 150] Ridgway et al. reached lower LODs with direct INCAT/SPDE compared to headspace in the extraction of furan and toluene of from water. [150] With direct SPDE the LODs were 0.64 µg/L and 0.17 µg/L for furan and toluene, respectively, while roughly double with headspace SPDE at 1.5 µg/L and 0.48 µg/L. Although direct INCAT/SPDE of extraction has not been examined to the same extent as headspace extraction, it can potentially be used to improve the extraction efficiency in relatively clean samples, such as water.

5.7 Desorption

Van Durme et al. investigated desorption of toluene from a PDMS sorbent thoroughly using three different desorption methods. [146] The method which gave the best response was a heating combined with a slow flow of helium through the needle. Even then, toluene required a minute in 250 °C to become fully desorbed from the 50 µm PDMS sorbent. Due the long desorption time, it was necessary to focus the desorbed toluene at the beginning of the analytical column with low temperature to obtain a good peak shapes. Slow desorption method has been recommended by other authors as well. [143, 145] It is possible to use repeated draw/eject cycles in emptying the needle from all adsorbed compounds if the focusing is effective enough. [139] In either case, as with the case of toluene, it was reported that desorption can result in a wide or split peaks in the chromatogram. Due to this reason, low column temperature before starting the chromatographic separation is recommended.

For highly volatile compounds focusing is not possible, as they will remain mobile in the column even in low oven temperatures. As an example, Jochmann et al. noted that especially early eluting, volatile compounds exhibit peak tailing even with a GC starting temperature of 40 °C, which was held for 10 minutes (Figure 29). [143] Although the higher boiling compounds had acceptable peak shapes, the low boiling compounds near the beginning of the chromatogram have increasingly poor peak shapes. The peak of the most volatile one, vinyl chloride (boiling point -13 °C), tails very badly.

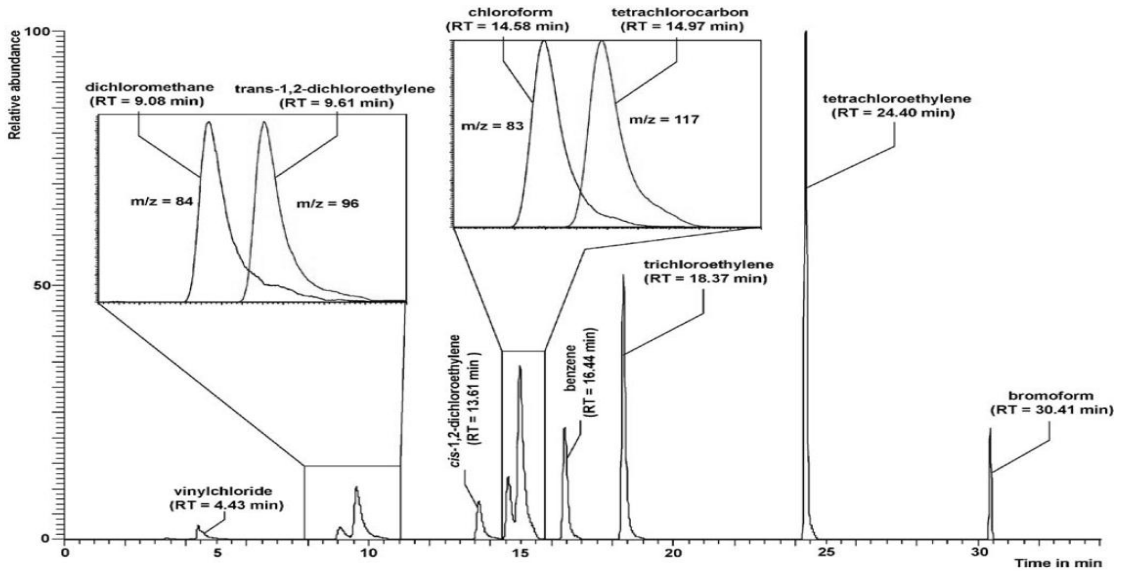

Figure 29. Highly volatile compounds can exhibit peak distortion even with low GC starting temperatures in INCAT/SPDE. Starting temperature was 40 °C with a hold of 10 minutes. 60 m x 0.32 mm RTX-VMS WCOT column with a film thickness of 1.8 µm, MS detection. Desorption 10 µL/s for 100 seconds in 300 °C. [143] Reprinted with permission from Springer Publishing.

Focusing of very low boiling compounds such as vinylchloride is only possible with a cryofocusing device capable of lowering column temperature significantly below room temperature. Therefore, analysis of low boiling compounds with INCAT/SPDE may be problematic without specialized equipment.

The volume of desorption gas is linked to the desorption speed. Larger amount of desorption gas is more effective for desorbing the extracted analytes, but it takes longer to flush it all through the needle, especially because low flow rate is usually recommended. Due to this reason, when it is possible to focus the analytes at the beginning of the column, large gas volumes have been reported to increase response. [145] When focusing is not possible, distorted or split peaks have occurred. [143] Naturally, depending on the matrix, analytes and sorbent, the desorption gas volume and flow rate will have to be optimized for that application. It is not recommended to use pre-desorption, meaning placing the needle in the injector and starting the gas flow later with INCAT/SPDE. Similar to other needle formats, the air inside the needle will heat and expand, flushing some of the desorbed analytes into the column before desorption gas flow starts. This may result in poor peaks shapes, especially for volatile compounds. [151, 152]

Like with other SPME methods such as TFME, which are coupled with GC and based on larger volumes of sorbent, desorption seems to be a critical issue in INCAT/SPDE. Careful attention should be paid to optimize desorption time and temperature during validation of an extraction method to ensure complete desorption. If possible, it would be even better to establish the desorption profile of all analytes.

5.8 Sorbent materials and coating processes

By directly coating the inside of a metal needle the problems of placing a silica capillary inside a needle are circumvented, but attaching sorbents on metal surfaces can also be problematic. Because INCAT/SPDE was commercialized soon after its introduction, many studies have used commercial needles. The coating process of these is not easily available. Currently, most commercial needles use PDMS, or PDMS-based sorbents.

McComb et al. deposited a carbon sorbent inside a needle by either drawing graphite paint in the needle and drying or by burning dichloromethane inside the needle. [67] It was noted that the graphite paint method deposited more carbon on the inside surface, while the dichloromethane burning method proved unsatisfactory. Lipinski, on the other hand, simply cut pieces of metal GC capillary with a PDMS stationary phase, straightened them and attached a Luer lock on the other end to use the pieces as needles. [68] Other GC column stationary phases have also been utilized

as INCAT/SPDE sorbents, [153] and can be produced relatively easily in the same manner. In the search for new sorbents, other deposition methods have also been experimented with. Djozan et al. silylated the inside of the metal needle to make it possible to polymerize a MIP sorbent on the inside wall. [139] Examples of electrochemical deposition [135] and sol-gel [58] can also be found. A more straightforward method was used by Gholivand et al., in which a nanocomposite material was immobilized with epoxy glue on the inside walls of a needle. [149] Currently, however, INCAT/SPDE lacks an effective and tested sorbent deposition method that would be suitable for many types of sorbent materials. Having to develop a new coating method for each sorbent limits the research into new materials.

5.9 Cooled solid phase dynamic extraction

An innovation used in several studies is the cooling of the needle. [142, 149, 154] This follows the same logic as cooling systems in other types of SPME: lowering the temperature of the sorbent material relative to the sample increases the distribution coefficient of the analytes, leading to higher enrichment levels on the sorbent.

Due to the fact that in most extraction systems only the tip of the INCAT/SPDE needle is in the sample vial, it is easier to construct a cooling system for it than for fiber-SPME or TFME, in which the whole sorbent is in the sample. In addition, in INCAT/SPDE the sorbent is attached to metal, which is a good thermal conductor, allowing for faster cooling and heating. There is a commercial device available for cooling the needle from Chromtech, but it has also been done with a self-made system that circulated cold water around the needle. [149]

An example of the effect of cooling was given by Rossbach et al. who compared the extraction efficiency of various volatile organic compounds in a water sample in 50 °C when the only variable was cooling to -15 °C (Figure 30). The peak areas for all compounds are dramatically increased when the needle is cooled. In headspace extraction, needle cooling can also allow heating the sample to higher temperatures which increases partitioning of compounds to the headspace. For example, Gholivand et al. noted that high temperature difference between the needle and sample increased amounts of extracted analytes. [149] Similar results have already been confirmed with fiber-SPME and TFME. [60]

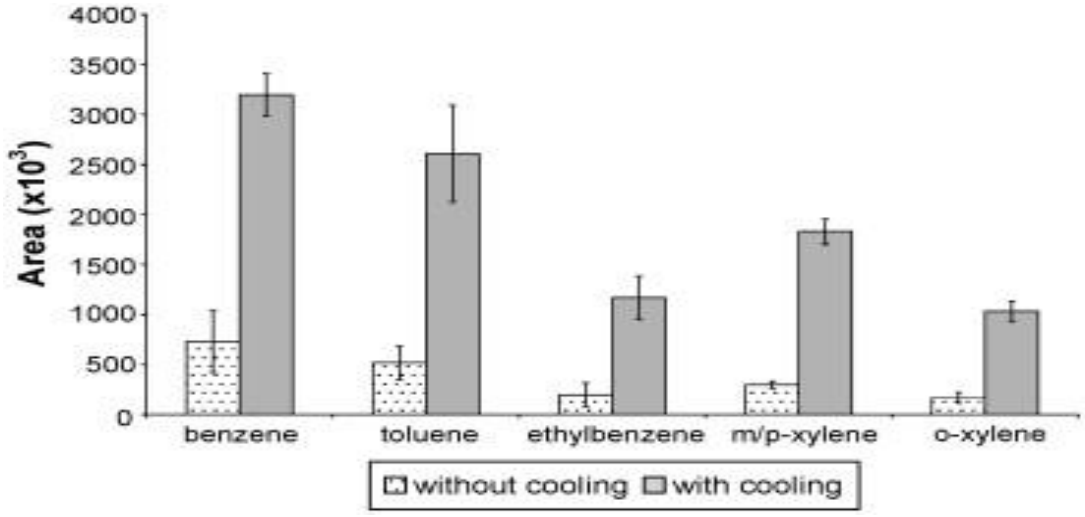

Figure 30. Comparison of GC-MS peak areas via solid-phase dynamic extraction of a spiked water sample with and without needle cooling. Sample temperature was 50 °C, cooling temperature was -15 °C. Concentration of all analytes was spiked to 1 μg/L, error bars represent relative standard deviation of three repetitions. [154] Reprinted with permission from Elsevier B.V.

5.10 Trends in in-tube capillary trap and solid phase dynamic extraction

INCAT/SPDE is still a new technique, and does not have a large body of research behind it. Different applications of INCAT/SPDE have been collected in Table 4. Similar to other newly introduced techniques, initially spiked water samples were the most common samples to establish the possibilities and limitations of the technique. In recent years, extraction of analytes from more complicated sample matrices, such as wine and blood or its components has also been done. As one might expect, INCAT/SPDE technique is mostly applied in extracting volatile compounds from the headspace of different matrices. As a headspace technique, it is also possible to extract compounds from solid matrices with less preparation than in IT-SPME, as was done in studies by Musshoff et al. [151, 155] and Lachenmeier et al. [156] However, other INCAT/SPDE applications for solid samples have not been introduced to my knowledge.

There is at least one example of field sampling with INCAT/SPDE. [148] Flow-through sampling requires only a portable pump and an adapter to connect the INCAT/SPDE needle. Therefore, INCAT/SPDE is quite simple to adapt to field sampling where complicated systems may not be available.

Table 4. Applications of INCAT/SPDE

Analyte	Matrix	Material	Analysis	Reference
Volatile organic compounds	Spiked water	Carbon	GC-FID	[67]
Organic compounds	Spiked water samples	PDMS	GC-MS	[68]
Amphetamine and its derivatives, cannabinoids	Washed hair samples	PDMS/activated carbon (on-sorbent derivatization)	GC-MS	[151, 155]
Drugs of abuse	Washed hair samples	PDMS/activated carbon (on-sorbent derivatization)	GC-MS/MS	[156]
Volatile organic compounds	Various plants and foods	PDMS/activated carbon	GC-MS	[145]
Polar volatile organic compounds	Spiked water samples	Poly(ethyleneglycol) WAX, cyanopropylphenyl/polydimethylsiloxane 1701, PDMS, PDMS/activated carbon	GC-MS	[153]
Insect pheromones and their precursors	Elephant secretions and excretions	Not stated	GC-MS	[157]
Benzene, toluene, ethylbenzene and xylenes	Soft drinks	PDMS and PDMS/activated carbon	GC-MS	[158]
d-limonene degradation products	Spiked water samples	PDMS	GC-FID	[144]
Hydrocarbons	Groundwater	PDMS/activated carbon	GC-MS	[143]
Toluene	Air	PDMS	GC-MS	[146]

Table 4 continues.

Analyte	Matrix	Material	Analysis	Reference
Volatile organic compounds	Melted snow	PDMS/activated carbon	GC-MS	[154, 159]
Gamma-hydroxybutyric acid	Blood serum, urine	Polyethyleneglycol WAX, cyanopropylphenyl/polydimethylsiloxane 1701, PDMS/activated carbon, 5%-phenyl-methylsiloxane	GC-MS	[160]
Volatile compounds	Fermenting grape must	PDMS/activated carbon	GC-MS	[141]
PAHs	Water	PPy-dodecyl sulfate	GC-MS	[135]
Desomorphine and desocodeine	Spiked water and urine	Sol-gel titania	GC-MS	[58]
Biogenic volatile organic compounds	Air	PDMS	GC-MS	[148]
Triazine herbicides	Spiked water	MIP	GC-FID	[139]
Polycyclic aromatic hydrocarbons	Spiked water	Polyaniline/hexagonally ordered silica nanocomposite	GC-MS	[149]
Volatile compounds	Red wines	Polyethyleneglycol	GC-MS	[147]
Volatile organic compounds	Elephant urine	PDMS/activated carbon	GC-MS	[161]
Volatile aroma compounds	Red and white wine	PDMS/activated carbon	GC-MS	[140]
n-heptane and its metabolites	Whole blood	PDMS/activated carbon	GC-MS	[142]

Table 4 continues.

Analyte	Matrix	Material	Analysis	Reference
Volatile compounds	Rat blood plasma	PDMS	GC-MS/MS	[162]
Volatile aroma compounds	Crab boiling juice	PDMS	GC-MS	[163]

Although INCAT/SPDE is still a relatively new technique, it has gained popularity because it offers larger sorbent volumes and better durability than fiber-SPME. Automation of INCAT/SPDE is somewhat more complex, because INCAT/SPDE as a dynamic technique requires pumping. Passive sampling is slow and not recommended. [67, 164] There are some examples of self-made autosamplers, [146] although most researchers have decided to use a commercial unit.

Currently, the main limitation of INCAT/SPDE is a lack variety in sorbent phases, as can be seen from Table 4. Similarly to TFME and IT-SPME, most initial work has been done with PDMS or PDMS-based sorbents. The limitations of PDMS as a sorbent material have already been discussed in chapter 3.5, and also apply to INCAT/SPDE. Only in recent years have there been reports of more novel extracting phases. [58, 135, 149] However, research into new sorbents is still in its infancy. Due to the possibility of modifying metal GC capillaries into INCAT/SPDE devices, [164] there is also potential to adapt more GC stationary phases into use as sorbent phases, although they have limitations as well, as discussed in chapter 4.6. Similar to most types of SPME, the extraction and desorption conditions need to be carefully controlled in order to obtain reproducible results. This is most repeatable with automated systems, which can increase the costs.

INCAT/SPDE also faces competition from other in-needle extraction techinques, such as needle trap and in-tube extraction, which work under very similar conditions. These packed needle devices have most of the advantages of INCAT/SPDE, but are capable of exhaustive extraction. For example, needle trap is outwardly a very similar extraction device to INCAT/SPDE, but offers exhaustive extraction and faster enrichment capability. Therefore, it is not surprising that most research attention has been focused on needle trap extraction. Since 2010, there have been 119 publications with the words “needle trap” in the abstract, while only 24 publications containing the words “inside needle capillary extraction” or “solid phase dynamic extraction” (CAplus database). It remains to be seen if INCAT/SPDE can carve out a niche in the increasingly crowded field of solventless extraction techniques.

6. Experimental part

The experimental portion of this thesis concerns the initial phases of method development for determination of small aliphatic amines from atmospheric air. Typical representatives of this group are amines such as methylamine, dimethylamine and trimethylamine. Although their concentrations in the atmosphere are low, [165] there is evidence that they play an important part in the formation of atmospheric aerosol. [166]

Despite of their importance, there is a lack of fast and effective methods to measure amines in atmospheric concentrations. Most current methods rely on time-consuming pre-concentration of amines on filters for particulate amines or denuders for gaseous amines. Due to the high pK_a's of the amines, they are often analyzed with ion chromatography, but response of most detectors is poor. In response to this need for new methods, an SPME on-fiber derivatization approach was applied to gaseous amine samples.

6.1 Derivatization in solid phase microextraction

Derivatization in SPME is done largerly for the same reason as derivatization in other types of analysis, for example to improve the chromatographic properties and detectability of analytes. [167] In SPME there can be further advantages if the K_D values of the derivatized forms are higher than those of the original compounds, such as better extraction efficiency. There are several ways to derivatize compounds in SPME:

(1) In the sample matrix (air) before extraction.

(2) On the fiber during extraction or after the extraction.

(3) After desorption into the chromatographic separation system.

This work focused on the second type, more specifically derivatization on-fiber during extraction (see Figure 31 for a representation on how the process works). In order to perform simultaneous derivatization and extraction, it is necessary to pre-load the derivatization reagent on the fiber before extraction (Phase 1 in Figure 31). After this, the fiber is moved into the sample container where the analytes are extracted and derivatized (Phase 2).

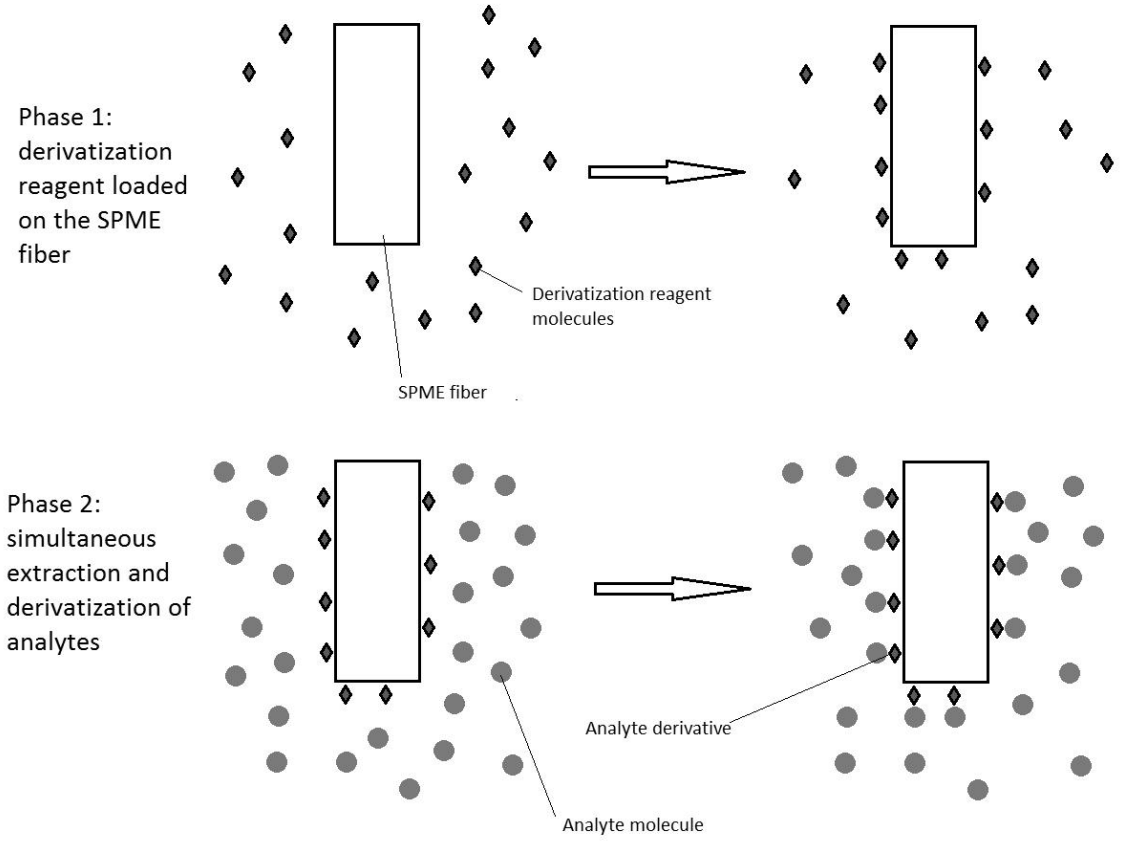

Figure 31. Pre-loading of the derivatization reagent (phase 1) followed by simultaenous extraction and derivatization (phase 2).

Simultaneous extraction and derivatization was chosen because it is suitable for derivatization of gaseous analytes. In comparison, derivatization of compounds in a completely gaseous sample is difficult unless the reagent is also gaseous. Furthermore, the analytes were all highly volatile, which generally results in low K_D value. This makes extraction of sufficient quantities of underivatized analytes unlikely without special arrangements, such as custom-made sorbents or cooled fiber. On the hand, adding a heavier moiety to small volatile analytes generally makes them less volatile, increasing their partition on the fiber.

6.2 Derivatization reagents

Three derivatization reagents were investigated in this work: allyl isothiocyanate, pentafluorobenzaldehyde and pentafluorobenzyl chloroformate. Their structural formulas can be seen in Figure 32.

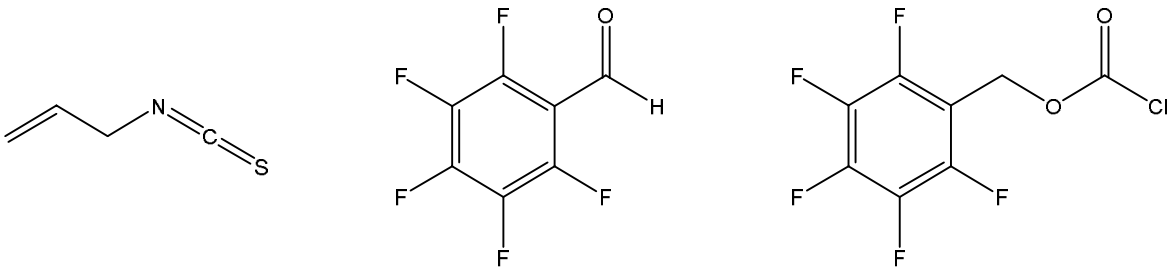

Figure 32. Structural formulas of the derivatization reagents investigated. From left to right: allyl isothiocyanate, pentafluorobenzaldehyde and pentafluorobenzyl chloroformate.

6.3 Allyl isothiocyanate

Allyl isothiocyanate had been previously used for on-fiber derivatization of aromatic primary amines. [168] It is a highly toxic compound with a strong pungent odor, and requires extra precautions to avoid skin contact or inhalation during handling. This complicates its use in the field, but as one of the few compounds with previous on-fiber application for derivatizing amines, it was included in the study.

Allyl isothiocyanate reacts with aromatic amines to form an intermediate derivative, which then pyrolyses inside the hot injector port of the GC, as shown in Figure 33, to produce the final form. [168]

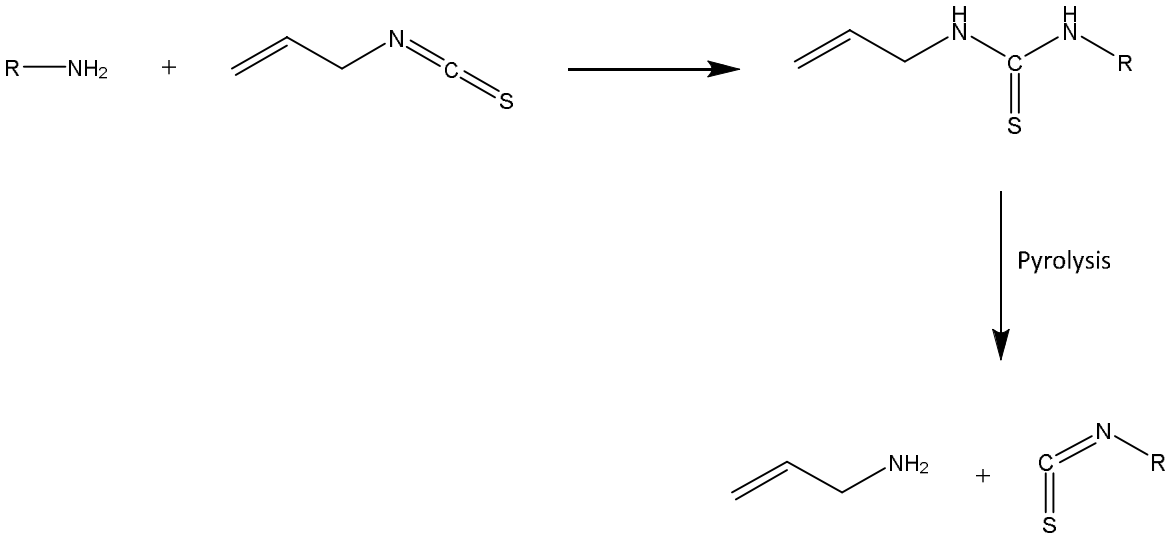

Figure 33. Reaction of allyl isothiocyanate with an aromatic primary amine followed by pyrolysis in GC injection port. R is any type of aromatic structure.

Despite its having confirmed information of derivatives forming with only aromatic primary amines, it was hoped that derivatization with allyl isothiocyanate could be extended for small, volatile aliphatic primary amines as well.

6.4 Pentafluorobenzaldehyde

Pentafluorobenzaldehyde (PFBAY) has a relatively long history as a derivatization reagent for primary amines. [169, 170] It was an attractive choice for this study, because it had already been used for on-fiber derivatization of amines of interest in this study, such as methylamine and ethylamine. [171, 172] In a previous study with on-fiber derivatization, with a 20 minute extraction and on-fiber derivatization time, detection limits of 70 μg/kg for ethylamine and 19 μg/kg for methylamine in sewage sludge samples after pressurized hot water extraction and analysis with GC-MS-MS were achieved. [171] These limits of detection are a few orders of magnitude higher than the estimated amine concentrations in air, so it was not known if on-fiber derivatization with PFBAY would be a viable method for their analysis.

PFBAY reacts by forming imines with primary amines (Figure 34). Due to the addition of the heavy pentafluorobenzyl moiety, PFBAY derivatives of small aliphatic amines are much less volatile than the underivatized compounds, increasing K_D. In addition, they are more thermally stable and possess better chromatographic properties, which make them easier to separate and detect.

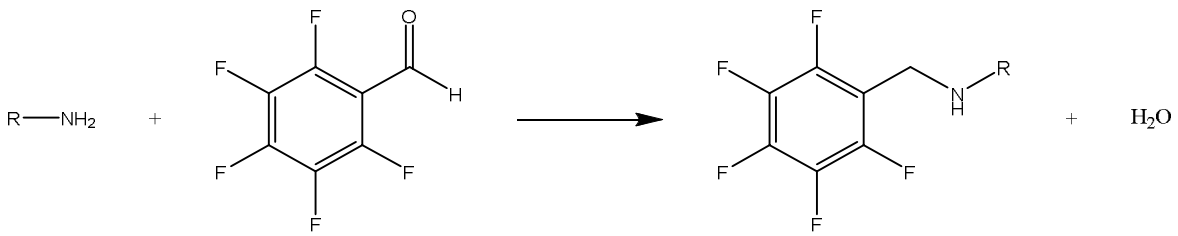

Figure 34. PFBAY reacting with a primary amine. R is any carbon chain.

PFBAY's main limitation is the same as with allyl isothiocyanate: it can only react with primary amines. This excludes amines which are of high interest in studying the formation of atmospheric aerosol, most important of which is dimethylamine.

6.5 Pentafluorobenzyl chloroformate

Pentafluorobenzyl chloroformate (PFBCF) belongs to a large group of chloroformate derivatization reagents, which form carbamates together with primary, secondary and tertiary amines. The reaction product of PFBCF with secondary amine is shown in Figure 35. Other chloroformates react in the same manner, with the structure of the derivatized form depending on the structure of the chloroformate.

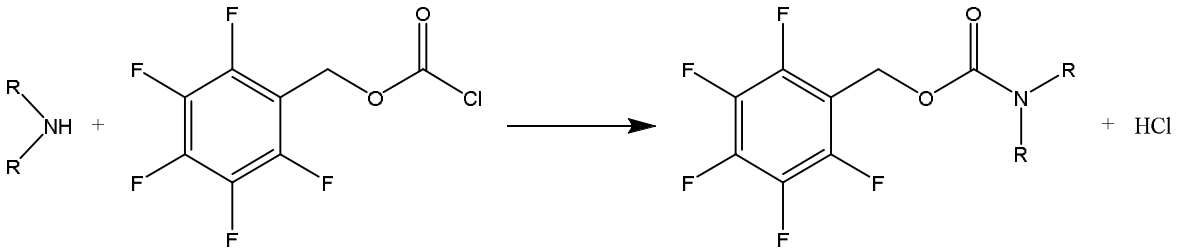

Figure 35. Reaction of PFBFC with a secondary amine. R is any carbon chain.

Similar to PFBAY, PFBCF and other chloroformates have long been known as a derivatization reagents for GC analysis [173] and there are studies available with different compound classes, including amines. [174, 175] In at least one instance, isobutyl chloroformate was used to derivatize small, aliphatic primary and secondary amines, which were the interest of this study. [176]

However, there was scarce information available for the feasibility of using any chloroformates for on-fiber derivatization of amines. To my knowledge, only one choloformate reagent has been previousy used for on-fiber derivatization of amines. [177] In this study the authors used 9-fluorenylmethyl chloroformate to derivatize trimethylamine in water samples for LC-MS determination.

PFBCF was chosen over isobutyl chloroformate, as isobutyl chloroformate is highly toxic and less stable in air owing to its high solubility in water. PFBCF on the other hand, is not as dangerous to handle and insoluble in water, which makes it more robust to store and use. Similar in structure to PFBAY, the pentafluorobenzyl moiety of PFBCF is heavy, thus decreasing the volatility of the analytes. One possible problem in using chloroformates for derivatizing atmospheric amines is that dimethylamine and trimethylamine may produce the same derivative as the final product of the reaction. [178] In addition, HCl forms as a byproduct of the amine-chloroformate reaction and can have a negative impact on the longevity of the fibers.

6.6 Standards and reagents

The reagents and solvents used in this work together with their purities and suppliers are listed in Table 5.

Table 5. Reagents and solvents used with their purities and suppliers.

Chemical	Purity	Supplier
Allyl isothiocyanate	≥93 %	Sigma-Aldrich (St. Louis, USA)
PFBAY	98 %	Sigma-Aldrich (St. Louis, USA)
PFBCF	Unavailable	Sigma-Aldrich (St. Louis, USA)
Dimethylamine-HCl	99 %	Sigma-Aldrich (St. Louis, USA)
Ethylamine-HCl	98 %	Sigma-Aldrich (St. Louis, USA)
Methylamine-HCl	98 %	Sigma-Aldrich (St. Louis, USA)
Ultrapure water		Millipore DirectQ-UV (Billerica, USA)
Acetonitrile	99.9 %	VWR International (Radnor, USA)
Dichloromethane	99 %	Fisher Chemical, (Loughborough, UK)
KOH	Unavailable	J.T. Baker (Sweden)

6.7 Gas chromatographic and mass spectrometric conditions

Throughout this study, Agilent 6890N gas chromatograph coupled with an Agilent 5793N quadrupole mass selective detector was used (Agilent Technologies, Palo Alto, USA). The column was a Zebron ZB-5MS (Phenomenex, Torrance, USA) with 5 % diphenyl 95 % dimethyl-polysiloxane stationary phase. Its dimensions were 30 m x 0.25 mm with a phase thickness of 0.25 μm. In

addition a 1 m x 0.53 mm deactivated fused silica retention gap was utilized in front of the analytical column.

All injecions were made in splitless mode through a Merlin microseal split/splitless injection port (Merlin Instrument Company, Half Moon Bay, USA). Helium was used as the carrier gas. With liquid standards, 1 μL was injected with a metal needle designed for use with Merlin microseal. Each reagent and derivative had their own GC oven program. They are shown in Table 6.

Table 6. GC oven programs used with each derivatization reagent and their derivatives.

Program	Allyl isothiocyanate	Pentafluorobenzaldehyde	Pentafluorobenzyl chloroformate
Starting temperature (hold)	60 °C (3 minutes)	50 °C (2 minutes)	50 °C
1st ramp	9 °C/minute	15 °C/minute	10 °C/minute
Temperature after 1st ramp (hold)	180 °C (0.5 minutes)	135 °C	130 °C
2nd ramp	20 °C/minute	20 °C/minute	100 °C/minutes
Final temperature (hold)	280 °C (2 minutes)	280 °C (2 minutes)	280 °C (2 minutes)

70 eV EI was used with all analytes. MS transfer line was maintained at 300 °C, the ion source temperature at 230 °C and quadrupole temperature at 150 °C. Quadrupole analyzer scan ranges were 45 to 450 m/z for allyl isothiocyanate and its derivatives, 40-450 m/z for PFBAY and its derivatives and 30-300 m/z for PFBCF and its derivatives.

6.8 Solid phase microextraction fibers and optimization of the extraction

Custodion™ (Torion Technologies, American Fork, USA) series manual SPME syringes were used in this study. The fibers were purchased from Supelco (Bellefonte, USA). Only PDMS/DVB coated silica fibers with a phase thickness of 65 μm were used.

The general workflow of the study with each reagent is shown in Figure 36. Before any actual on-fiber derivatization and extraction of amines could be studied, it was first necessary to optimize the loading of the fiber with the derivatization reagents. In order to do this, however, it is also necessary to know the desorption characteristics of the reagent. The desorption properties of the pure reagents was also applied to the derivatives.

Firstly, a calibration curve with the reagent dissolved in GC-compatible solvent was made. From this curve, it was possible to determine the amounts of reagent desorbed from the SPME fiber, allowing for the study of desorption and loading times in amounts and repeatability. After optimal method of loading and desorption was found, it was possible to study the on-fiber derivatization process. Most important of part of this was to study the approximate LODs with each reagent, as aliphatic amines are present in trace concentrations in the atmosphere.

The loading procedure should be sufficiently repeatable to load a substantial excess of derivatization reagent on all fibers to successfully derivatize amines present in air. To ensure this, the variation between the loaded amounts of the reagents was studied between the fibers.

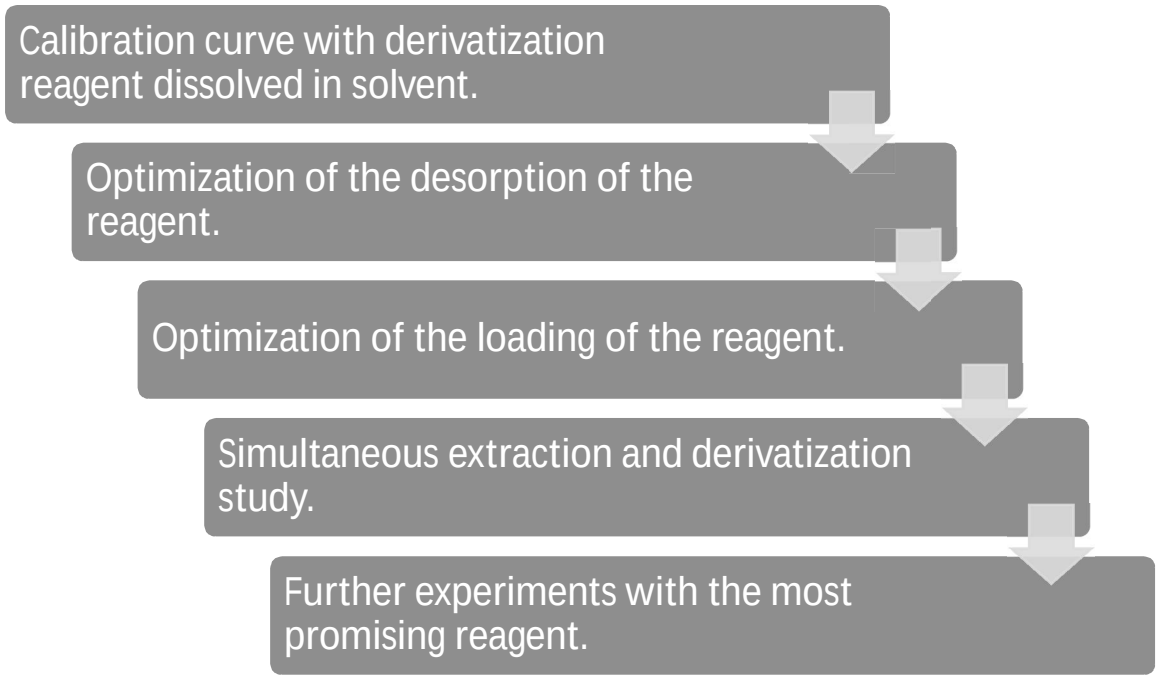

Figure 36. Workflow for each investigated derivatization reagent. Last step only with one reagent.

6.9 Simultaneous extraction and on-fiber derivatization

Unfortunately, there was no gaseous amine available to be diluted into a gas phase standard. Therefore, the process shown in Figure 37 was used to generate gaseous amine standards from water solutions of amines. Firstly 1 mL of amine standard solution was pipetted inside a 1.2 L Erlenmeyer flask, which was then covered with Parafilm. 0.1 mL of 5 M KOH was then pipetted through the Parafilm with a metal needle and the solutions mixed so they covered the bottom as a film. After this the released amine was extracted with a derivatization reagent-loaded SPME fiber.

Since all the analytes were gases at room temperature, it was assumed that they would be nearly quantitavely released from the water solution if it was made sufficiently basic by the addition of 5 M KOH. However, since the water remains in the Erlenmeyer flask throughout the extraction it is possible that some of the amine may remain dissolved. Furthermore, more seriously, due to the holes in the Parafilm some of the gaseous amines may diffuse out of the flask. Therefore, this method of generating gaseous amine standard must be considered as having fairly high uncertainty.

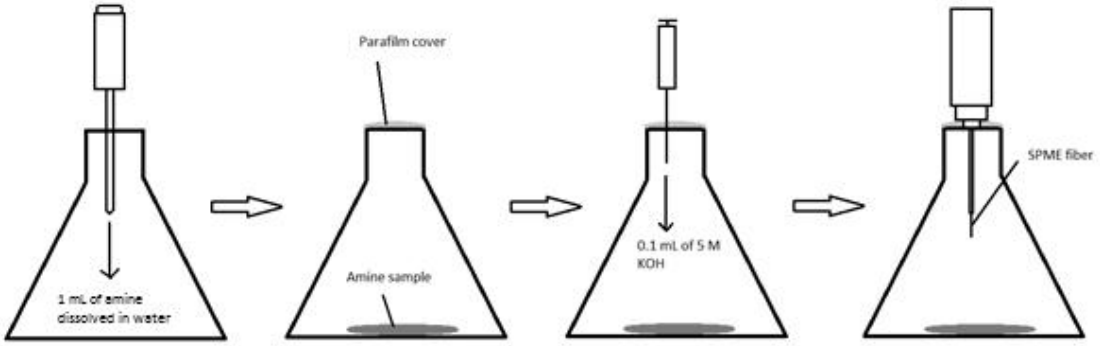

Figure 37. Method of generating gaseous amine standards and their extraction with a derivatization reagent-loaded fiber.

7. Results with allyl isothiocyanate

7.1 Fiber loading procedure with allyl isothiocyanate

Loading of allyl isothiocyanate was the most straightforward to test, as it already had an established procedure. [168] In this approach, the fiber was loaded in headspace of a 4 mL glass vial with 2 mL of 3 % v/v allyl isothiocyanate solution in acetonitrile. Loading time was 10 minutes at 70 °C. Unfortunately, there was no information available on how much allyl isothiocyanate was loaded on the fiber in this manner, so it had to be determined. Moreover, it was also decided to attempt loading by directly immersing the fiber into the coating solution, as this is usually faster due to reagent concentration being higher in solution than in headspace.

Derivatization reagent was dissolved in acetonitrile and desired amount was pipetted into a 20 ml glass vial sealed with a screw cap equipped with a rubber septum. The metal needle protecting the SPME fiber was used to penetrate through the septum and the fiber was exposed in the headspace of the vial.

The amount of allyl isothiocyanate loaded on the fiber was determined from allyl isothiocyanate calibration curve (see Appendix 1). To make this, a primary standard of 143 mg/L allyl isothiocyanate in dichloromethane was further diluted with dichloromethane. Throughout the experiments, ion with m/z 99 was used to quantitate allyl isothiocyanate.

7.2 Desorption time

Due to the need for quantitative pyrolysis of the primary derivative in the injection port, in the previous study desorption time was extremely long, 5 minutes in 250 °C in splitless mode. [168] However, desorption time for unreacted allyl isothiocyanate was not available and had to be experimentally determined.

Desorption times of 10, 20 and 30 seconds with a generic 10 minute fiber loading in 70 °C was studied with four different fibers (Figure 38). It was found out that injection time did not have a large influence on the amount of allyl isothiocyanate detected. Taking repeatability into account the amount was the same with all desorption times. Finally, for the loading time study 20 second desorption was chosen as it had a slightly better repeatability than 10 or 30 second desorption.

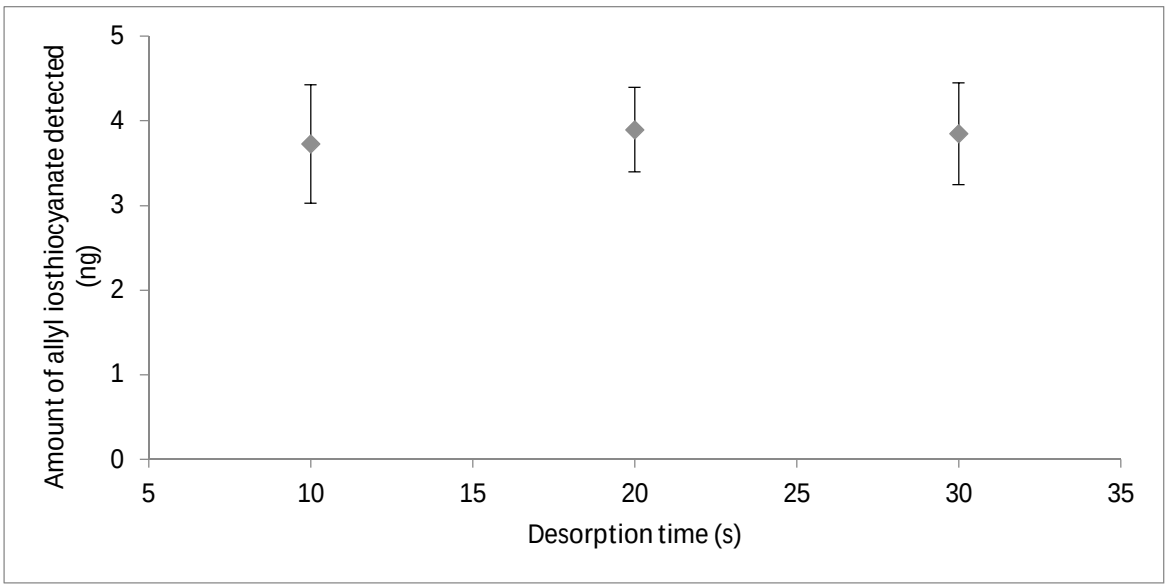

Figure 38. Amount of allyl isothiocyanate detected with GC-MS using 10, 20 and 30 second desorption times from SPME fiber. Inector temperature was kept in 250 °C. Loading time was 10 minutes in 70 °C. Error bars represent the standard deviation between five different fibers.

7.3 Allyl isothiocyanate loading time

The amounts of allyl isothiocyanate loaded on the fibers while varying the loading time was determined from the calibration curve. The extraction profile of allyl isothiocyanate is shown in Figure 39.

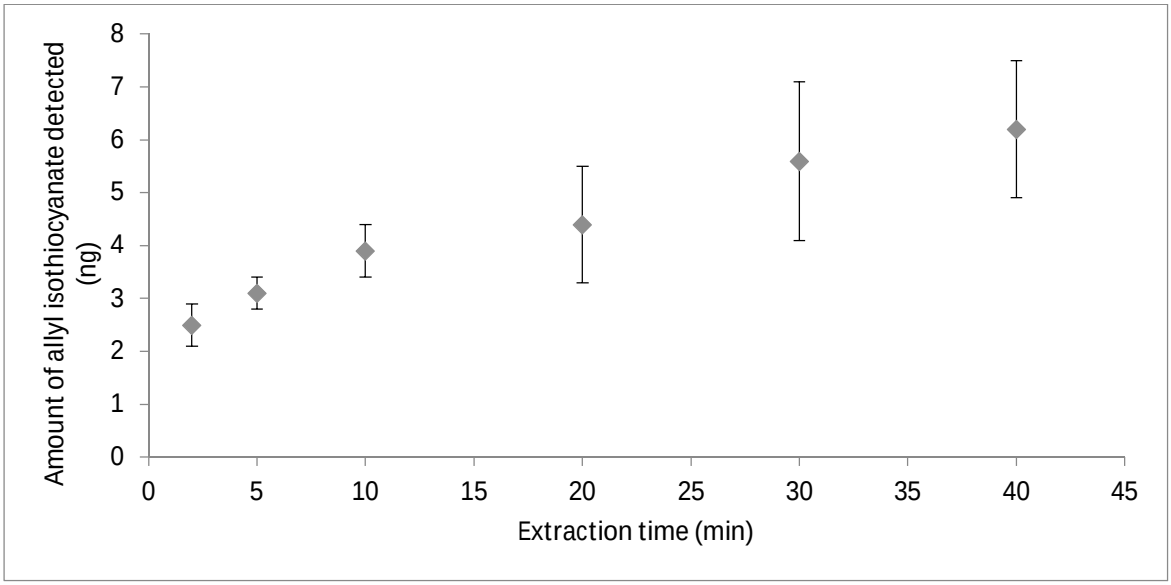

Figure 39. Loading time profile of allyl isothiocyanate in 70 °C with PDMS/DVB SPME fibers. Desorption time 20 seconds in 250 °C. Error bars represent the standard deviation between five different fibers.

As expected, longer loading time increased the amount of allyl isothiocyanate on the fiber. With the loading time of 5 minutes used in the previous study, an average of 4 ng of allyl isothiocyanate was loaded on the fibers. It can be seen from Figure 39 that with 5 minutes loading time we are still in the linear regime of the extraction profile, as the linear regime seems to end between 10 and 20 minutes. Therefore, the greatest gains in the amount of allyl isothiocyanate that can be achieved by increasing loading time are within the first 20 minutes. After this, the loading kinetics slow down and also the inter-fiber repeatability seems to decrease.

There were some differences with the experiments to Sharma et al. They had used 4 mL vials, [168] but 20 mL vials were used in this study. However, the headspace concentrations should be the same, as partitioning between liquid and gaseous phases is an equilibrium process. It should also be mentioned the fiber material used by Sharma et al. was PDMS/CAR/DVB, [168] but in this study PDMS/DVB was used instead.

7.4 Direct immersion loading

Because directly immersing the fiber in a solution will also load higher amounts of solvent on the fiber, it was decided to use dichloromethane as a solvent instead of acetonitrile in order to avoid damaging the GC column. A 3 % v/v allyl isothiocynate solution was made in dichloromethane and 10 mL of it was pipetted into a 20 mL headspace bottle sealed with a septum screw cap. A much larger volume of 10 mL loading solution compared to headspace loading had to be used in order to immerse the fiber fully.

Initially, much higher mass of allyl isothiocyanate was observed on the fibers. However, after a few injections it was noted that dichloromethane tends to swell the PDMS/DVB-coated SPME fibers. If a swollen fiber was withdrawn back to the metal needle, the coating was damaged and in some cases completely destroyed as the metal needle peeled the coating from the silica fiber. It may be possible to use direct immersion loading in dichloromethane with fiber materials which do not swell in organic solvents, but such materials were not available in this study and direct immersion loading had to be abandoned.

7.5 Extraction and on-fiber derivatization experiments

As mentioned, the PDMS/CAR/DVB fibers that were chosen as optimal in the previous study [168] were not available in this research. Although Sharma et al. tested other fiber materials as well, unfortunately PDMS/DVB was not among those tested. However, as PDMS/DVB fibers were the only ones available for this study, it was decided to attempt derivatization and extraction on these.

Sharma et al. had reached limits of detection of 6-160 ng/L for aromatic amines dissolved in water, which have significantly lower vapor pressures than the small aliphatic amines for which the current method was being developed. A much higher concentration 10 mg/L ethylamine standard was made, which would generate a headspace concentration of about 8.3 µg/L using the method shown in Figure 32.

Loading time was initially 5 minutes and extraction and on-fiber derivatization time 20 minutes, which was shown by Sharma et al. to result in maximum conversion of amines into the allyl isothiocyanate derivatives for aromatic amines. [168] With the injection/pyrolysis time of 5 minutes used by Sharma et al. no ethylamine derivative was detected in three repeated determinations. Loading time was later changed to 10 and finally 20 minutes, and the extraction time to 40 minutes, but ethylamine derivative was not detected in any of the experiments. Finally ethylamine from a 100 mg/L water solution was extracted (headspace concentration about 83 µg/L), but even then allyl isothiocyanate derivative of ethylamine was not found. It should be noted that in all of the

cases the byproduct of the pyrolysis reaction, allylamine (see Figure 33), was not detected either. From this one could conclude that either allyl isothiocyanate did not react with ethylamine or that its product did not undergo pyrolysis in the injector port of the GC.

As time was limited, it was decided to cease work on allyl isothiocyanate and move on to the two other derivatization reagents. The exact reason why on-fiber derivatization with allyl isothiocyanate did not work was not resolved due to the multitude of factors that can affect the analysis, such as loading, pyrolysis and difference in analytes. For the time being, allyl isotiocyanate can only be confirmed working for aromatic primary amines using the method described by Sharma et al.

8. Results with pentafluorobenzaldehyde

8.1 Fiber loading procedure with pentafluorobenzaldehyde

Fiber loading and desorption were optimized in a different work. [179] Briefly, the optimal loading conditions were 5 minutes in headspace with the temperature of 70 °C. Similar to allyl isothiocyanate loading, 2 mL of loading solution (1 mg/mL of PFBAY in acetonitrile) was pipetted to a 20 mL septum capped headspace vial. For loading, the metal needle was punctured through the septum and the fiber exposed in the headspace of the vial. The injection time was 20 seconds and GC injection port temperature 250 °C. With this method, the cross-fiber average of PFBAY loaded was roughly 40 ng.

8.2 Headspace extraction and derivatization of amines with pentafluorobenzaldehyde

The extraction and on-fiber derivatization experiments were made according to the procedure shown in Figure 36. Methylamine (2088 mg/L) and ethylamine (2258 mg/L) primary standards were made by dissolving HCl salts of the amines to water. These were further diluted with water to obtain the working standards. Fragment m/z 208 was used in drawing the extracted ion chromatograms.

Initial experiments were made separately with 100 mg/L solutions of ethylamine and methylamine, which would have a gas phase concentrations of about 83 µg/L after the addition of KOH. Extraction/on-fiber derivatization time was 20 minutes, which was used in the previous study. [172]

A peak for ethylamine PFBAY derivative was seen at 6.30 minutes (Figure 40). However, after diluting the ethylamine solution to 10 mg/L, no peak was detected at this retention time with three

repetitions despite. 40 minute and 60 minute extraction times were also tested with the same 10 mg/L concentration, but ethylamine derivative was not detected at 10 mg/L with longer times either.

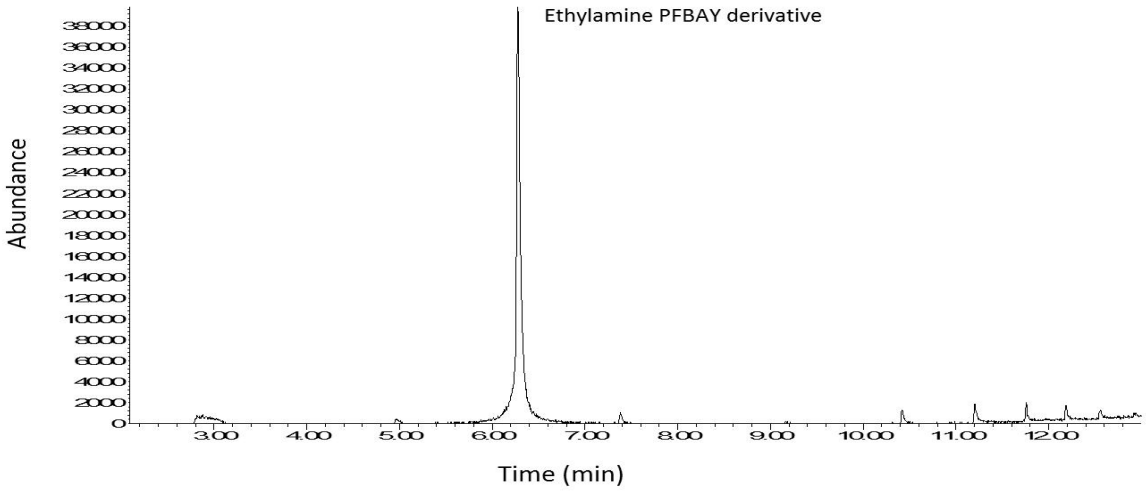

Figure 40. Extracted ion chromatogram with m/z 208. 1 mL of 100 mg/L ethylamine solution volatilized with 100 µL of 5 M KOH extracted for 20 minutes with a PFBAY-loaded PDMS/DVB fiber in room temperature. Desorption time was 20 seconds and temperature 250 °C.

No peak for methylamine PFBAY derivative was detected even with 100 mg/L solution, so 1 mL of undiluted primary standard (concentration 2088 mg/L) was used instead. In this case, the headspace concentration was extremely high, 1.7 mg/L, in comparison to atmospheric levels. With this high concentration a strong peak was detected at 5.30 minutes (Figure 41).

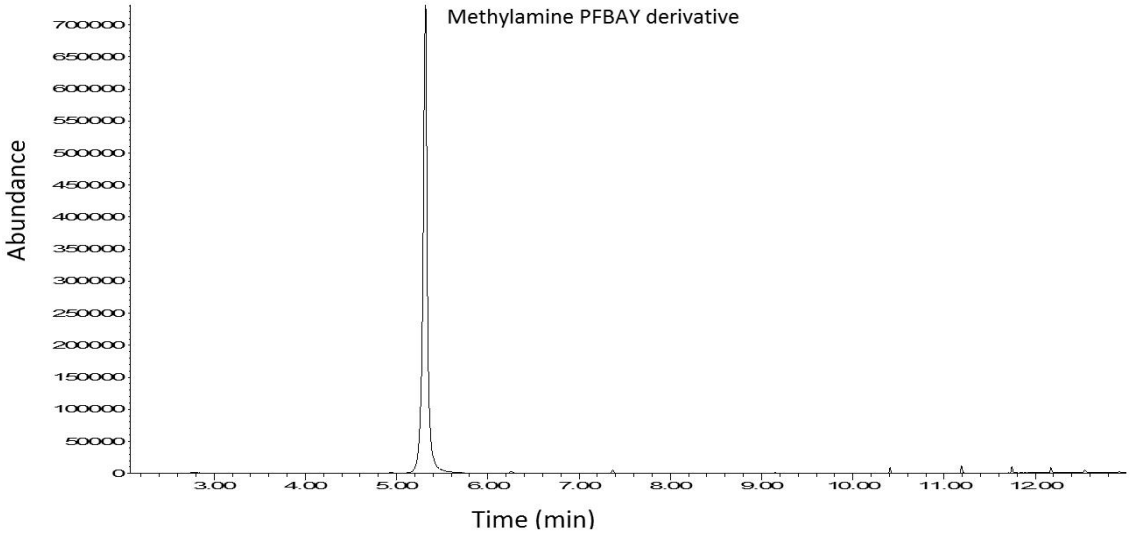

Figure 41. Extracted ion chromatogram with m/z 208. 1 mL of 2088 mg/L methylamine solution volatilized with 100 µL of 5 M KOH and extracted for 20 minutes with a PFBAY-loaded PDMS/DVB fiber. Desorption time was 20 seconds in temperature of 250 °C

Based on these results, PFBAY can derivatize on-fiber both ethylamine and methylamine, confirming the previous results. [172] However, the limits of detection limit in the gas phase fall somewhere between 8.3 μg/L and 83 μg/L for ethylamine and over 83 μg/L for methylamine. These limits of detection are clearly unacceptably high considering the typical atmospheric concentrations of these amines, which are estimated to be in the nanograms per liter level. [165]

Previous study with pressurized hot water extraction and GC-MS-MS had established limits of detection in sewage sludge at 70 μg/kg for ethylamine and 19 μg/kg methylamine. [171] However, in this study the limit of detection was much higher for methylamine than for ethylamine. The reason for this may be different fibers – previously 85 μm polyacrylate fibers and 65 μm PDMS/DVB fibers in the current study.

PFBAY was found to be unsuitable for the purpose of determining atmospheric amines. While already having the serious drawback of only derivatizing primary amines, the detection limits were not low enough to determine amines in ambient air.

9. Results with pentafluorobenzyl chloroformate

9.1 Fiber loading procedure with pentafluorobenzyl chloroformate

Out of the tree tested derivatization reagents, PFBCF was the only one without any known history of use for on-fiber derivatization. Therefore, it was necessary to develop a fiber loading and on-fiber derivatization and extraction procedures from the beginning.

A limited amount of 100 mg of PFBCF was available, so only a small amount of loading solution could be prepared. In order to avoid SPME fiber swelling and damage, it was decided to use acetonitrile as the solvent in making the loading solution, as it had worked well as a solvent with allyl isothiocyanate and PFBAY.

Similarly with studies on the previous two reagents, the workflow shown in Figure 36 was followed. In the quantification of pure PFBCF, ion with m/z 260 was used. Calibration curve with pure PFBCF is shown in Appendix 2.

First, it was necessary to simply see if PFBCF could be successfully extracted from its acetonitrile solution, and whether concentration would have a large effect on the extracted amount. In these

initial experiments, neither desorption time nor temperature had been studied, so generic 20 second injections at 250 °C were used.

Initially 2 mL of 0.4 mg/mL PFBCF was tested with the extraction times of 5, 10 and 15 minutes in 30 °C, but no PFBCF was detected in any of the tests. Moving on to 2 mL of 2 mg/mL PFBCF in acetonitrile provided a peak with the same molecular mass as PFBCF with extraction time of 5 minutes in 30 °C. However, the amount of PFBCF proved unsatisfactory at less than 2 ng.

SPME is an equilibrium-based extraction technique. The concentration, not the total mass of compound, is one of the primary factors controlling the amount of compound extracted. It was reasonable to assume that a small volume of highly concentrated PFBCF should result in higher amount of PFBCF loaded on the fiber.

Following this reasoning, 1 mL of 10 mg/mL acetonitrile solution was prepared, of which 0.5 mL was deposited into a headspace bottle. With 5 minute extraction time in 30 °C, about 20 ng of PFBCF was loaded on the fiber. A comparison of chromatograms with 2 mg/mL and 10 mg/mL PFBCF solutions can be seen in Figure 42. Following the results of this experiment, 10 mg/mL solution was considered suitable for further studies with loading time and temperature.

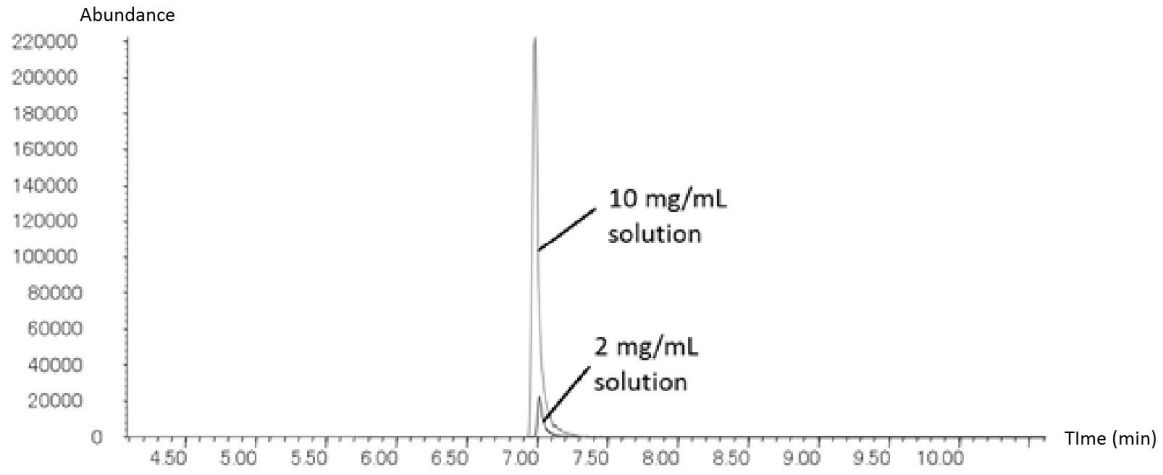

Figure 42. Overlayed exctracted ion chromatograms with m/z 260. Each PFBCF solution extracted with PDMS/DVB SPME fiber for 5 minutes in 30 °C. Injection time 20 seconds in 250 °C.

9.2 Desorption time optimization

Because the loading process was not optimized at this point, a preliminary 10 minute loading time in 30 °C was used. Three injections with 5, 10, 20 and 30 second desorption time were made using

the same fiber in the temperature of 250 °C. The results were compared to establish optimal desorption time. The results can be seen in Figure 43.

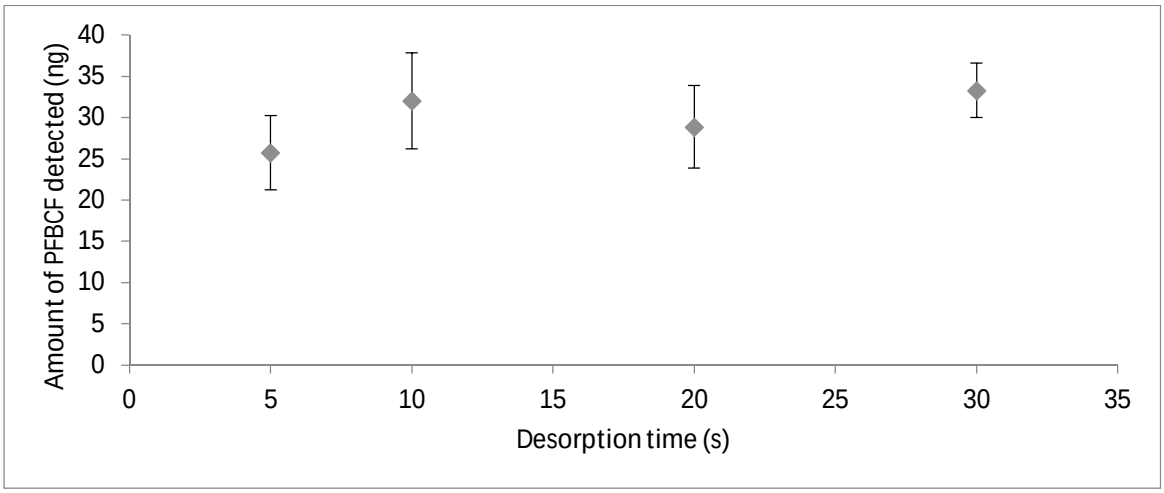

Figure 43. Effect of desorption time on the amount of PFBCF detected. PFBCF was loaded 10 minutes in 30 °C prior to desorption in 250 °C. Error bars represent standard deviation within three repetitions.

The amounts detected were the same when uncertainty was taken into account. However, the repeatability with the 30 second desorption was slightly better than with other desorption times. Therefore, 30 seconds was selected as the optimal desorption time in all following experiments.

9.3 Desorption temperature optimization

It was not known how PFBCF would behave in the injector port of the GC, which is typically kept at temperatures between 150 °C to 300 °C. If PFBCF is thermolabile, lowering the GC injection port temperature should increase the amount of PFBCF detected. On the other hand, at lower temperatures PFBCF may not fully desorb from the SPME fiber, especially with shorter desorption time. This would reduce the amount of PFBCF detected or increase the variation in the detected amounts. To test this if temperature would have an effect on the amount of PFBCF detected three injections in both 200 °C and 250 °C were made with the same fiber.

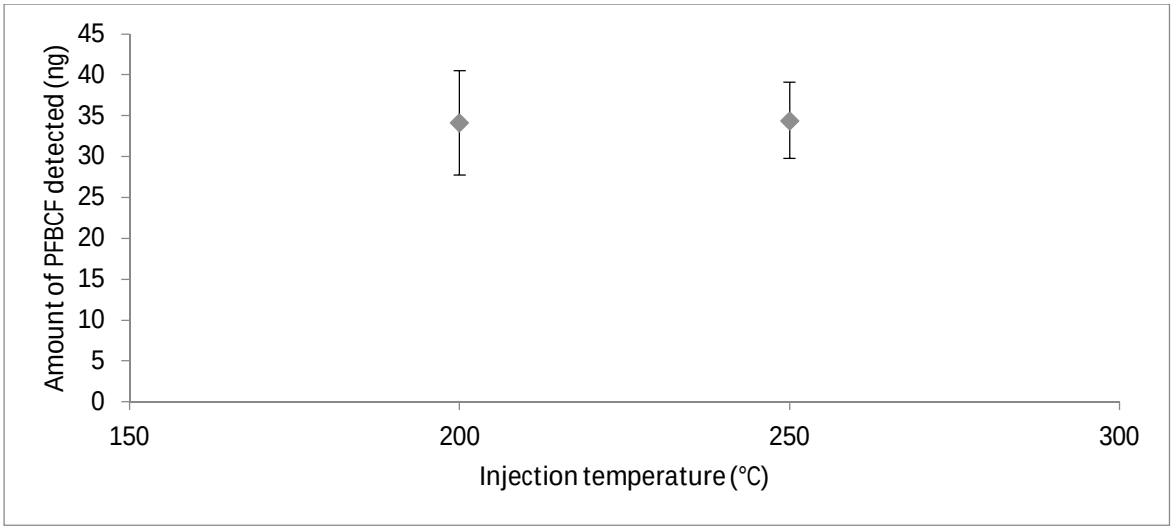

Figure 44. Effect of injector port temperature on the amount of PFBCF detected. PFBCF was loaded 10 minutes in 30 °C prior to desorption. Desorption time was 30 seconds in both temperatures. Error bars represent standard deviation within three repetitions.

At 250 °C, repeatability was slightly better, but otherwise temperature did not seem to have a large effect on the amount of PFBCF detected.

Although it would have been interesting to see if higher desorption temperature would increase the repeatability further, it was decided not to test higher temperatures in order to preserve the fibers. According to the manufacturer, the PDMS/DVB fibers had a recommended conditioning temperature of 250 °C and a maximum operating temperature of 270 °C. Therefore, 250 °C represents a temperature where the fibers are relatively stable.

9.4 Degradation of reagent

Relatively soon after experimenting with higher loading temperatures, it was noticed that PFBCF degrades in its acetonitrile solution, as the initially satisfactory peak areas for PFBCF decreased with repeated fiber loading cycles. An experiment aimed at finding out how long the loading solution remains usable was made. 25 mL of 20 ng/µL PFBCF acetonitrile solution was made out of which four aliquots of 5 mL was taken. They were placed in temperatures of 30, 40, 50, and 70 °C in a heating block. After 20.5 h 1 mL was taken out of each aliquot and dissolved into 10 mL of dichloromethane. 1 µL of this solution was then immediately injected into GC to determine the concentration of PFBCF. The results are shown in Table 7.

Table 7. Degradation of PFBCF in 20.5 h in different temperatures as determined with GC-MS. Original concentration was 20 ng/µL.

Temperature (°C)	Peak area	Concentration (ng/µL)	Degradation
30	2205824	18.7	6.7 %
40	1965443	16.6	16.9 %
50	168990	1.4	92.9 %
70	Not detected	-	-

The degradation of PFBCF acetonitrile solution was faster in higher temperatures and increased quickly after room temperature. Already in the temperature of 50 °C, almost all of the PFBCF had degraded in 20.5 hours. It follows that only very mild temperature could be used in loading the fibers. Based on the results, the only reasonable temperature would be 30 °C, and it was selected as the loading temperature. Even at this temperature, the peak area of PFBCF had to be carefully followed.

9.5 Loading time

First it was thought that PFBCF loading would be studied by comparing the average loaded amount on different fibers. At this point, however, the fibers were in various states of use and the amounts of loaded PFBCF varied considerably between fibers depending on how and how much they had been used. The standard deviations between fibers were therefore very large, which made comparing each fiber individually more sensible. The comparison is shown in Figure 45. Especially Fiber 1 had an extremely high amount of PFBCF loaded on the fiber compared to the others even with very short loading times. On the other hand, fibers 2, 3 and 5 had less than 30 ng loaded on them even in 20 minutes, which was the longest loading time tested. Fiber 4 fell somewhere in the middle between the two extremes.

As a conclusion of the loading time tests, when using multiple fibers, it is important to test loaded amounts on each fiber one is going to use. This is especially true for used fibers. As can be seen in Figure 46, variation between fibers can be nearly 80 % with the longest loading times.

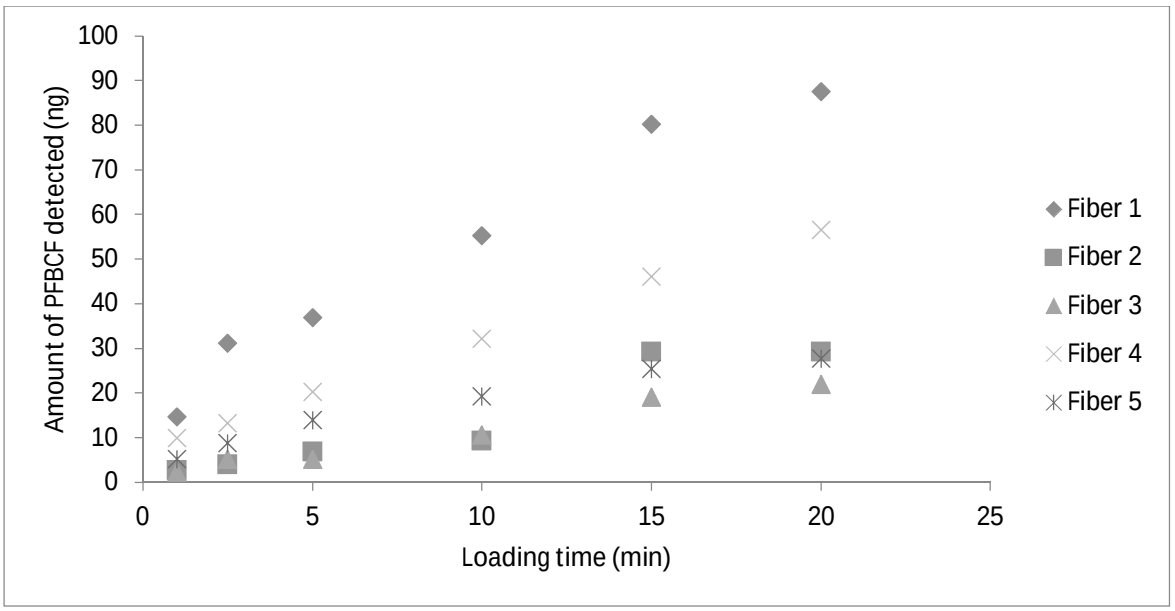

Figure 45. Comparison of loading time profiles for different PDMS/DVB fibers. Desorption was 30 seconds in 250 °C with all fibers, loading in 30 °C.

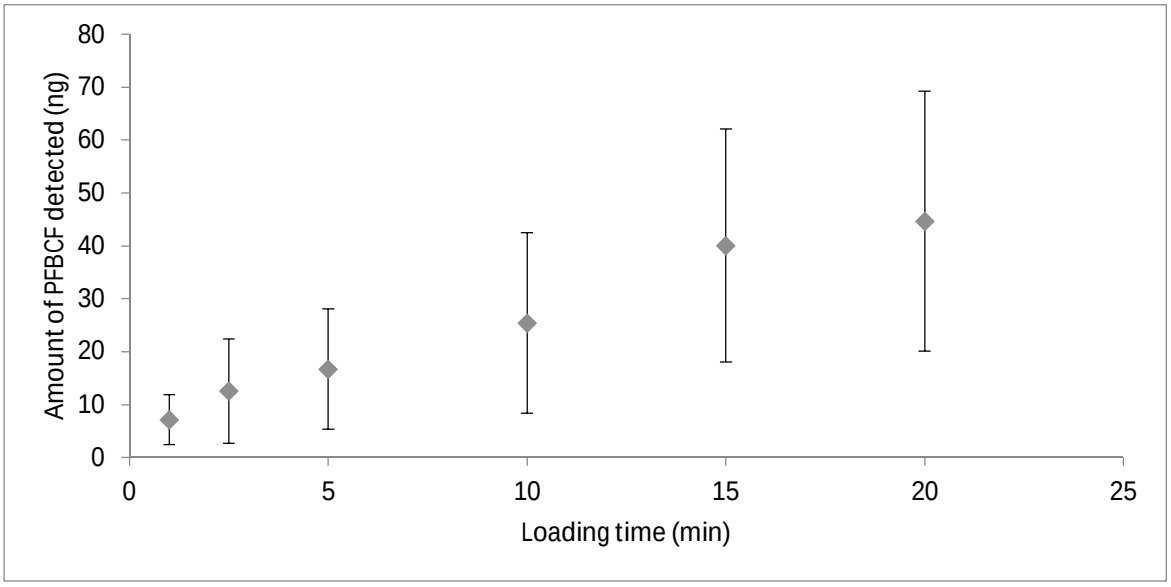

Figure 46. Average inter-fiber PFBCF amounts. Error bars represent standard deviation among five different PDMS/DVB fibers.

9.6 Determining the amounts of derivatives extracted

This last phase of experiments was done approximately two months later than the preceding studies. It should also be noted that during this time the fibers had been used for unknown quantities.

Solid dimethylamine-HCl and ethylamine-HCl were dissolved in ultrapure water in volumetric flasks. 100 µL of these solutions were further diluted in acetonitrile to make 143.2 mg/L and 139.8 mg/L

primary standard solutions, respectively. Approximately 2 mL of these solutions were then transferred to separate test tubes and 5 µL of pure PFBCF was pipetted to each of the tubes. Test tubes were then left sealed overnight in room temperature. Standard curve was made by diluting these solutions with dichloromethane. In the calculations, it was assumed that all of the amines had been converted to derivatized forms. No underivatized amines were detected, but it should be noted that the small aliphatic amine analytes had very little retention in the GC column used and detecting them would have been difficult even if present in small quantities.

In choosing the quantification ion, it was decided to use the molecular ion of each amine PFBCF derivative, although the fragment at m/z 181 is the base peak of the mass spectrum (see Figure 47) and produces the most intense peak. However, this fragment can be found in all peaks containing the pentafluorobenzyl-CH_2 moiety, including all derivatives and the reagent itself. Overlapping peaks may therefore become a problem in samples contains many amines. On the other hand, molecular ion is unique for each amine PFBCF derivative, excluding those amines which have the same molecular mass. The calibration curve for ethylamine PFBCF derivative is shown in Figure 48 and dimethylamine PFBCF derivative in Figure 49.

Figure 47. Mass spectrum of dimethylamine PFBCF derivative. Fragment m/z 181 representing the pentafluorobenzyl moiety is clearly the most intense.

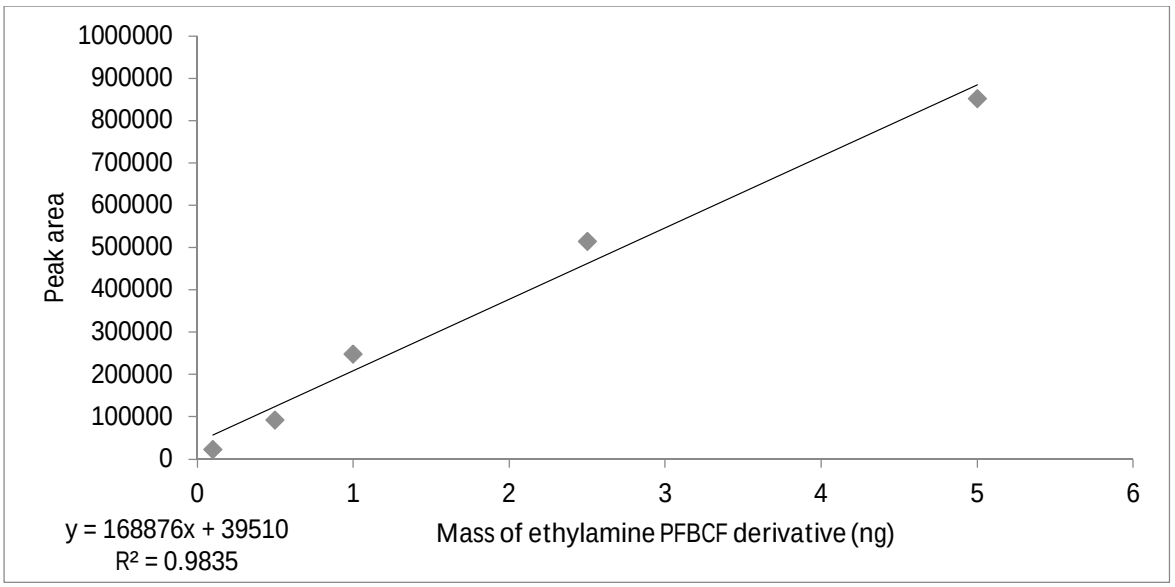

Figure 48. Calibration curve of ethylamine PFBCF derivative. Derivatization performed overnight in acetonitrile in room temperature with an excess of PFBCF. After dilution with dichloromethane 1 µL of liquid was injected to GC-MS. Peak area quantified with ion m/z 269.

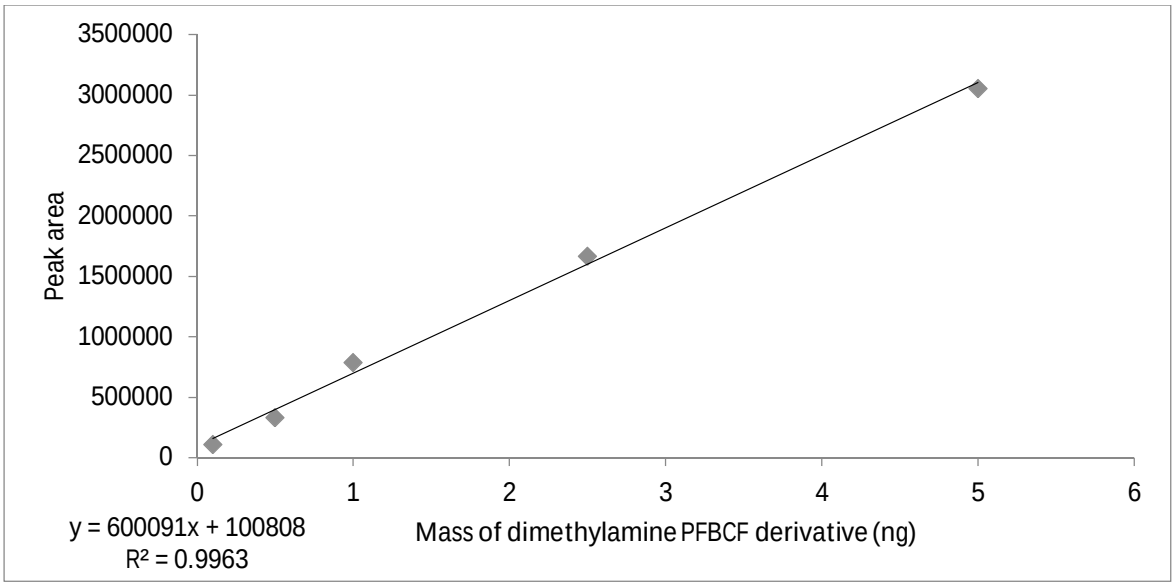

Figure 49. Calibration curve of dimethylamine PFBCF derivative. Derivatization performed overnight in acetonitrile in room temperature with an excess of PFBCF. After dilution with dichloromethane 1 µL of liquid was injected to GC-MS. Peak area quantified with ion m/z 269.

9.7 Headspace extraction and on-fiber derivatization of dimethylamine

A dimethylamine primary standard was prepared in ultrapure water by weighing solid dimethylamine-HCl salt. The primary standard was then diluted further with water to make the working standards.

Initial experiment with on-fiber derivatization and extraction of dimethylamine was made with 100 mg/L dimethylamine standard solution, which produced an intense peak as shown in Figure 50 with 10 minute extraction and on-fiber derivatization time.

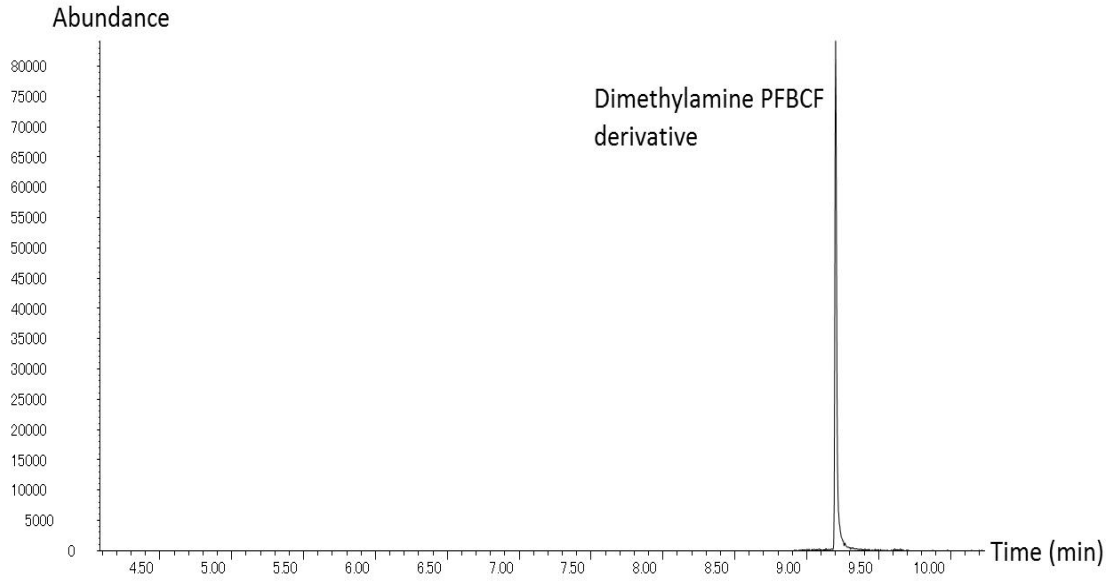

Figure 50. Extracted ion chromatogram with m/z 269. Sample was 100 mg/L aqueous dimethylamine, which was headspace extracted and derivatized on-fiber for time 10 minutes in room temperature with a PFBCF-loaded PDMS/DVB fiber. Desorption 30 seconds in 250 °C.

Due to the strong response, it was decided to make a headspace calibration curve in lower concentrations of dimethylamine. The headspace calibration curve is shown in Figure 51.

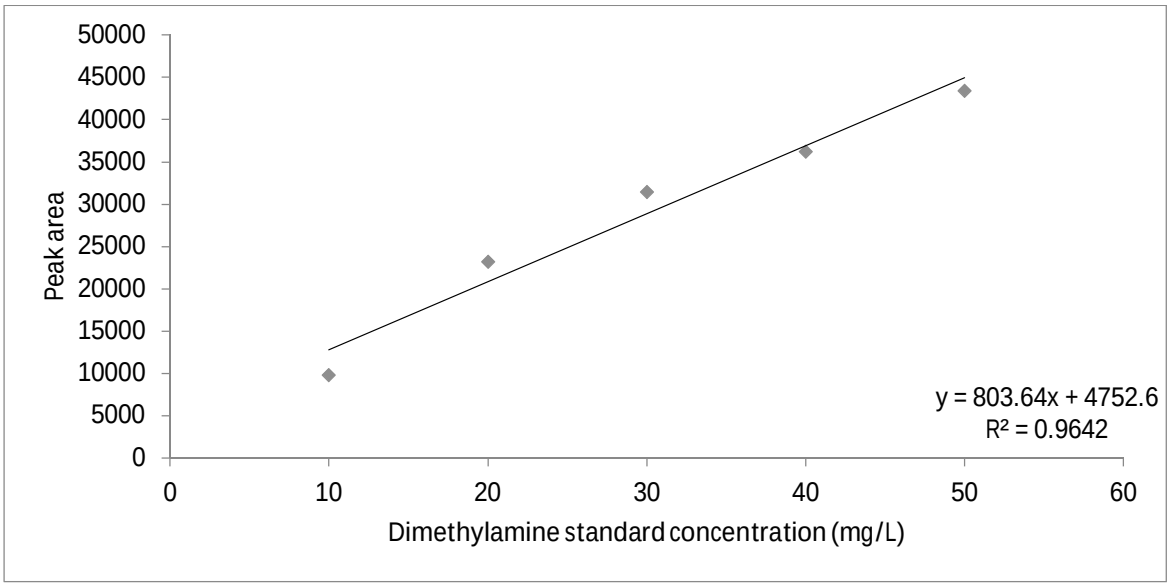

Figure 51. Dimethylamine headspace extraction and derivatization calibration curve. In each point headspace extraction and derivatization for 10 minutes with a PFBCF-loaded PDMS/DVB fiber. Desorption 30 seconds in 250 °C.

By comparing the peak areas of the headspace calibration curve of dimethylamine shown in Figure 51 to the calibration curve made with liquid standards in Figure 49, it is possible to estimate the mass of dimethylamine PFBCF derivative on the fiber, as shown in Table 8.

Table 8. Estimated masses of dimethylamine PFBCF derivative in headspace calibration.

Standard concentration (mg/L)	Approximate gas phase concentration (μg/L)	Mass of PFBCF derivative (ng)
50	42	0.56
40	30	0.44
30	25	0.36
20	17	0.22
10	8.3	Not quantifiable

It should be noted that the masses shown in Table 8 are highly approximate, as peak area values in all headspace standards are in the low end of the liquid calibration curve. The peak area in the most dilute headspace standard (10 mg/L) fell below the calibration curve in Figure 49 and was therefore not quantifiable.

Although full evaluation of uncertainty was not made, three points (50, 30 and 10 mg/L) from the calibration curve were repeated on a different day. It was discovered that the repeatability was very poor, giving relative standard deviations of 52, 45 and 71 percent, respectively.

9.8 Headspace extraction and on-fiber derivatization of ethylamine

Headspace extraction of 1 mL of 100 mg/L ethylamine standard produced a peak with an appropriate mass spectrum for a PFBCF derivative, which is shown in Figure 52. It is worth noting that it has longer retention than the dimethylamine PFBCF derivative. Therefore, they can be separated even though the masses are the same.

Although headspace calibration was attempted in a similar manner to dimethylamine, only the most concentrated 50 mg/L standard produced a clearly detectable peak at m/z 269. The vapor pressure of ethylamine is approximately 120 kPa at 20 °C, while vapor pressure of dimethylamine is 170 kPa. Therefore, the large difference in the peak intensities cannot be explained with the difference in vapor pressures alone. Instead, it may be concluded that ethylamine reacts more slowly with PFBCF than dimethylamine.

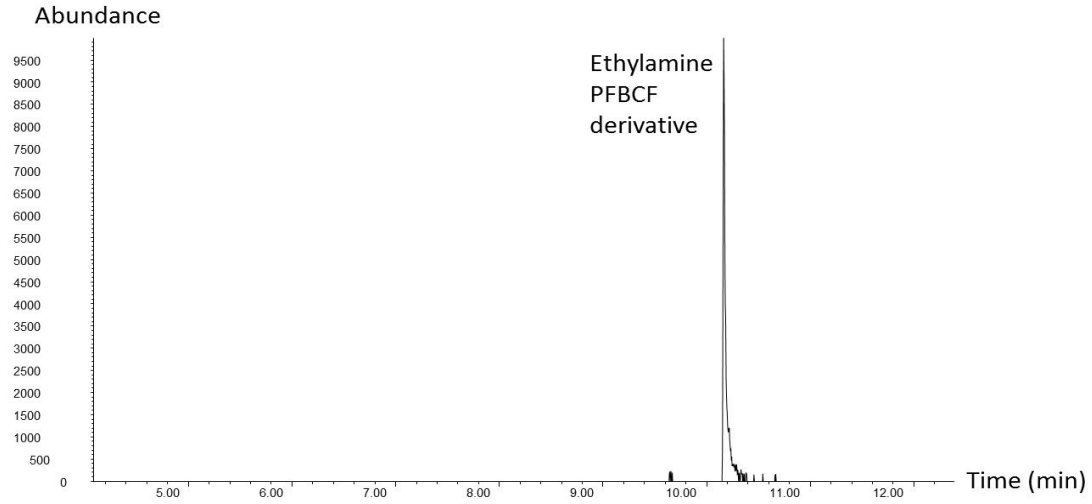

Figure 52. Extracted ion chromatogram with m/z 269. Sample was 100 mg/L aqueous ethylamine, which was headspace extracted and derivatized on-fiber for time 10 minutes in room temperature with a PFBCF-loaded PDMS/DVB fiber. Desorption 30 seconds in 250 °C.

9.9 Headspace extraction and on-fiber derivatization of amine mixture

Final phase of the study with PFBCF was to attempt a derivatization of a mixture of dimethylamine and ethylamine. Unfortunately, due to lack of time, only very limited preliminary trials could be made. A chromatogram of a mixture of dimethylamine and ethylamine is shown in Figure 53. These results also confirm the conclusion drawn earlier: dimethylamine appears to have much faster reaction rate with PFBCF than ethylamine, resulting in a more intense peak. Therefore, the limits of detection may be expected to be lower for dimethylamine. However, a full extraction profile for both analytes should be made to confirm this.

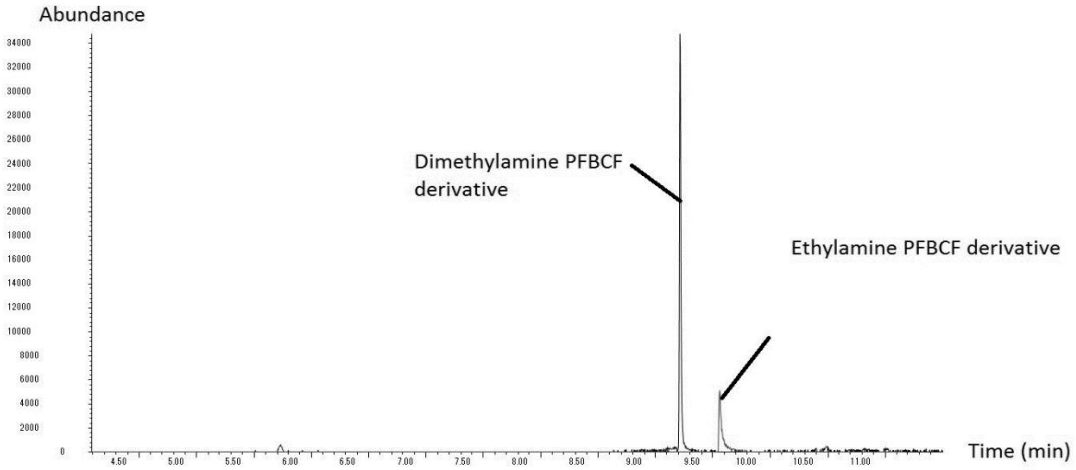

Figure 53. Extracted ion chromatogram with m/z 269. Sample was 1 mL of 50 mg/L aqueous mixture of both dimethylamine and ethylamine. Extracted and derivatized 10 minutes with a PFBCF-loaded PDMS/DVB fiber. Desorption 30 seconds in 250 °C.

10. Conclusions

In the theoretical part several new formats of SPME were reviewed. All have advantages and disadvantages compared to fiber-SPME.

TFME has optimal geometry for passive SPME sampling, and supports several types of calibration. As the style of operation is very similar as fiber-SPME, many of the methods developed for fiber can be relatively easily adopted to TFME. Thin films have faster sample uptake and larger capacity than fiber, but the large physical size presents problems in desorption, especially with GC. Currently the only material suitable for GC is PDMS, which imposes limitations on the analytes which can be extracted. Due to this, most of new research in TFME have been done with a coupling to LC instead. With development of new coating methods and materials, TFME seems to be maturing as a technique. However, due to the problems in coupling TFME with GC, it is unlikely to replace fiber-SPME in the near future.

IT-SPME is a dynamic type of SPME that can be easily automated with standard LC six-port autosampler valve. However, combining it with other analysis methods such as GC or CE has difficulties which have not been fully addressed yet. There are many types of extraction capillaries available in the form of commercial GC capillary stationary phases. In addition, many studies which describe self-made wall coated or packed extraction capillaries are available. As IT-SPME was introduced nearly 20 years ago, it has a substantial body of research behind it, and some more recently developed types are capable of very high levels of pre-concentration, all the way up to exhaustive extraction. Similarly to fiber-SPME, in recent years much focus has been put into developing new sorbent materials, which expand the usability of IT-SPME even further. Sorbent materials which respond to environmental factors such as temperature, magnetic fields or electric current seem especially promising.

INCAT/SPDE is likely to have the most uncertain future out of the techniques reviewed, although it has some advantages over fiber-SPME, such as larger sorbent volume and durability. It shares similar operation and automation to exhaustive techniques like needle trap and in-tube extraction, but cannot provide similar pre-concentration capability or capacity. As a dynamic extraction method, however, INCAT/SPDE still requires additional equipment compared to passive extraction devices such as fiber-SPME, increasing the complexity of automation. Moreover, there are technical difficulties in coating metal surfaces with sorbent material, as opposed to, for example, needle trap which is simply packed with sorbent particles. Therefore, not much research has been done towards

new sorbents unlike with TFME and IT-SPME. Currently, the sorbent selection is mainly limited to PDMS and PDMS-based materials.

In the experimental part of this thesis, PFBCF was found to be the most promising reagent for the on-fiber derivatization of atmospheric low molecular weight amines. It was possible to extract and derivatize ethylamine and dimethylamine separately and simultaneously, although the speed of the reaction with primary amines seems to be much slower than with secondary amines. Moreover, a calibration curve for dimethylamine was made by extracting the headspace gas of a sample. The main problem with PFBCF in the current stage of the study was its degradation in the fiber loading solution, which necessitated constant monitoring of the amount loaded on the fiber.

While it was possible to load the fibers with allyl isothiocyanate, simultaneous extraction and derivatization of gaseous ethylamine did not work even in very high ethylamine headspace concentrations. With a PFBAY-loaded fiber, on-fiber derivatization of methylamine and ethylamine was successful. However, the amine concentrations required to reach detectable amounts of the derivative were too high for the intended application.

There are many questions in loading and extraction with PFBCF that were not yet addressed by this study. For example, desorption temperature and time were only tested with PFBCF, not derivatized amines. Because so much time was spent on optimizing the loading procedure, there was little time to optimize the more important extraction and on-fiber derivatization. Therefore, the current procedure can be considered only tentative. The longevity of PFBCF and its amine derivatives on the fibers is still relatively unknown.

Drawing from the experiences in this work, it would be recommended in future studies with on-fiber derivatization to establish the coating procedure relatively quickly and move on to actual experimentation with extraction and on-fiber derivatization. Using very high concentrations or even pure substance in the fiber loading process seem to work more reliably than dilute solutions, and makes it faster to load sufficient amounts of reagents on fiber.

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Appendix

1. Calibration curve of allyl isothiocyanate.

2. Calibration curve of PFBCF.

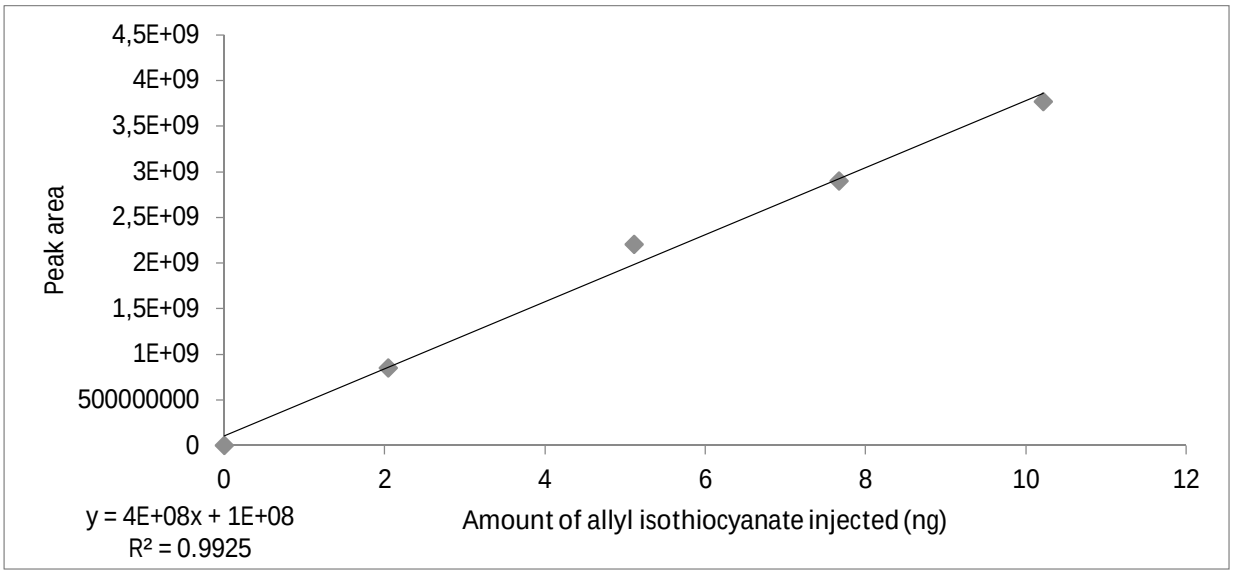

Figure 54. Calibration curve of allyl isothiocyanate. 1 µL of allyl iosthiocyanate dichloromethane solution injected.

Appendix 2. Calibration curve of PFBCF.

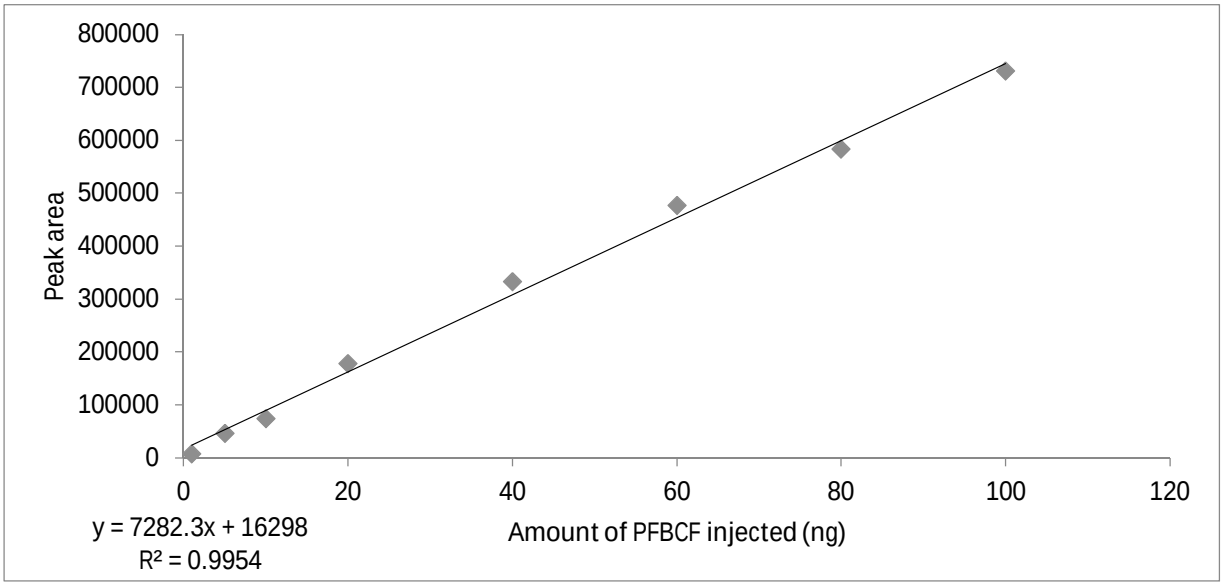

Figure 55. Calibration curve of PFBCF. 1 µL of PFBCF dichloromethane solution injected.